L’invention concerne des fragments isolés d’une protéine ou d’un polypeptide dérivés de Erwinia, élicitant une réaction d’hypersensibilité chez les plantes. L’invention porte en outre sur des molécules d’ADN isolées codant ce fragment d’Erwinia éliciteur de réaction d’hypersensibilité. Les fragments isolés de protéines ou de polypeptides éliciteurs de réaction d’hypersensibilité, qui élicitent une réaction d’hypersensibilité, ainsi que les molécules d’ADN isolées qui les codent peuvent être utilisées pour rendre les plantes résistantes aux maladies, pour améliorer la croissance des plantes et/ou pour lutter contre les insectes sur les plantes. A ces fins, on applique sous une forme non infectieuse des fragments éliciteurs de réaction d’hypersensibilité aux plantes ou aux graines de plantes dans des condition aptes à susciter une résistance à la maladie, à stimuler la croissance des plantes et/ou à lutter contre les insectes sur les plantes ou sur les plantes issues des graines. D’autre part, cette invention concerne des plantes transgéniques ou des graines de plantes transformées avec une molécules d’ADN codant le fragment éliciteur de réaction d’hypersensibilité, les plantes transgéniques ou les plantes issues de graines transgéniques étant cultivées dans des conditions aptes à conférer une résistance aux maladies, à stimuler la croissance des plantes et/ou à lutter contre les insectes sur les plantes ou sur les plantes issues de ces graines.
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(57) Abstract

The present invention is directed to isolated fragments of an Erwinia hypersensitive response elicitor protein or polypeptide which fragments elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode the Erwinia hypersensitive response eliciting fragment. Isolated fragments of hypersensitive response elicitor proteins or polypeptides, which elicit a hypersensitive response, and the isolated DNA molecules that encode them can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects on plants. This can be achieved by applying the hypersensitive response eliciting fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding a hypersensitive response eliciting fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.
HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS
ELICITING A HYPERSENSITIVE RESPONSE AND USES THEREOF

This application claims benefit of U.S. Patent Application Serial No. 60/048,109.

FIELD OF THE INVENTION

The present invention relates to fragments of a hypersensitive response elicitor which fragments elicit a hypersensitive response and uses thereof.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.


Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.


The first of these proteins was discovered in E. amylovora Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, “Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora,” Science 257:85-88 (1992)). Mutations in the encoding hrpN gene revealed that harpin is required for E. amylovora to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The P. solanacearum GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. “PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of Pseudomonas solanacearum,” EMBO J. 13:543-53 (1994)). However, P. solanacearum popA mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.


The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments elicit a hypersensitive response, and uses of such fragments.
SUMMARY OF THE INVENTION

The present invention is directed to an isolated fragment of an *Erwinia* hypersensitive response elicitor protein or polypeptide where the fragment elicits a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide which fragments elicit a hypersensitive response in plants and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a deletion and proteolysis analysis for the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin). A is the name of the harpin fragment. B is the length of the fragment in amino acid residues. C indicates whether detectable protein is produced. D states whether there is hypersensitive response (i.e., HR) eliciting activity. The solid line indicates that there are additional amino acids which are not harpin encoded, while the dashed line indicates the portion of the harpin that is
deleted. The numbers above the fragments in the box represent the amino acid residue present at the end of a given fragment; residue #1 is the N-terminus, and residue #403 is the C-terminus.

Figure 2 is a Western blot illustrating specific secretion of harpin_{Ea}, but not harpin_{Ea}C31. Lane A, Ea273(pGP1-2) CFEP; lane B, Ea273(pGP1-2)(pCPP1104) CFEP; lane C, E. coli DH5α (pCPP1107) CFEP harpin size standard; lane D, BioRad low range molecular weight markers; lane E, Ea273(pGP1-2) supernatant; lane F, Ea273(pGP1-2)(pCPP1104) supernatant. The blot was probed with an anti-harpin_{Ea} polyclonal antibody.

Figure 3 is an HR assay on tobacco leaf infiltrated as follows: (1) A, harpin_{Ea} + raspberry IF; (2) B, harpin_{Ea} + apple IF; (3) C, harpin_{Ea} + tobacco IF; (4) D, harpin_{Ea} + endoproteinase Glu-C; (5) E, harpin_{Ea} + trypsin; (6) F, harpin_{Ea}; (7) G, tobacco IF; (8) H, endoproteinase Glu-C; (9) I, trypsin; and (10) J, harpin_{Ea}. IF refers to intracellular fluids.

Figure 4 shows the digestion of harpin with endoproteinase Glu-C. Lane A is harpin; Lane B is harpin + endoproteinase Glu-C; Lane C is BioRad low range molecular weight markers.

Figure 5A shows the proteolysis of harpin. Coomassie blue stained polyacrylamide gel was loaded as follows: A, BioRad low range molecular weight markers; B, IF-apple; C, IF-raspberry; D, IF-tobacco; E, harpin_{Ea}; F, harpin_{Ea} + IF-apple; G, harpin_{Ea} + IF-raspberry; H, harpin_{Ea} + IF-tobacco.

Figure 5B shows a Coomassie Blue stained polyacrylamide gel loaded as follows: A, IF-tobacco; B, IF-tobacco + harpin_{Ea}; C, harpin_{Ea}; D, BioRad low range molecular weight markers; E, IF-tobacco + harpin_{Ea} + PMSF. HR-eliciting activity of the sample following proteolysis is denoted below the gel.

Figure 5C depicts whether proteolytic activity is present in IF from all plants tested. Intercellular fluid harvested from several plants was analyzed by PAGE in a gel containing 0.1% copolymerized gelatin. After washing to remove SDS and incubation to allow proteolysis of gelatin, the gels were stained to demonstrate the presence of gelatinolytic activity. A, IF-apple; B, IF-tobacco; C, IF-cotoneaster; D, BioRad mw; E, endoproteinase Glu-C; and F, ground leaf extract-tobacco.
Figure 6 shows the refractionation of elicitor-active peptides following proteolysis of harpin_{EA} by tobacco IF. Absorbance was measured at 210 nm. Peak 1 contains peptides P91 and P95; peak 2 contains peptides P65 and P69.

Figure 7 shows the predicted proteolytic cleavage sites within harpin of several tested proteinases, and the effect of these cleavages on activity of active harpin fragments. Residues potentially important for HR-eliciting activity, based on the loss of activity following further cleavage, are indicated by upward-pointing arrows at bottom.

Figure 8 shows the similarities near N-termini among harpins of *Erwinia* spp. Underlined residues are present (identical or similar) in at least four out of the five proteins examined. Nine out of the first 26 residues are conserved in this manner.


Figure 10 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

Figure 11 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments elicit a hypersensitive response in plants. Also disclosed are DNA molecules encoding such fragments as
well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia, Pseudomonas*, and *Xanthamonas* species (e.g., the following bacteria: *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris*, and mixes thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

```
  Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
  1       5       10       15
  Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
  20      25
  Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
  30      40
  Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
  45      55      60
  Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Glu Leu Gly Gln Ser
  65      70      75      80
  Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
  85      90
  Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
  95      100     105     110
  Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Glu Ser Asn Gln
  110     115     120     125
```
This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

```
30
CGATTTTACC CGGCTGGGC CAGCACTAC ACCAAGCGA CACGTTTACC
CGGTTTATGG CGCGATGAA CGGCGATCAG GCGCCGCGCT GGTGCCGCGA ATCCCGCGTC
```

35
GATCTGGTAT TTCACTTTGG GGACACCGGG CGTGAACTCA TGATAGCAGAT TCAGCCGGGG 180
CAGCAATATC CCGGCAAGTT GGCACGCTG CTGCTCGCTG TTATAGCAGCA GGGCAGCAAG 240
TGGCAAGGCT GCCAACATTG CCTGAACCGG AGCCGATATAT TGATCTCTTG GTGCGCGCTG 300
CCGTCGCAATC CCGGGCGTAA TCCGCAGGTT ATCGAACCGT TTCGTTGAAC GGGGCGAATG 360
ACGTTGCGGT CGCTACGTGA AGCAGCCCCG GCGGTCGCCG AGACAGGGAA CGGACGGGCC 420
CGATCTGAAA GATAAAGCGG GCTTTTCTTTA TGCTAAAACG GTAACGCTGA GGAACGGTTT 480
CACGGTCCGC GTGACATGTCA AACAAGTATC CATGATGTAG CTCATACTGG GATCCGGCGT 540
GGCATTGGTT GCGGACGACT TTGGCAAAACG CTGACATGAA TGGGAAAGC AAATATGCA 600
AATTACGATC AAAGCGGACA TCGCGGGGTA TGGGGCGCTC TCCGGTCTGG GCCTGGGTGC 660
TCAGGGACTG AAAGAGCTGA ATTCGCGGCG TGGATCGCTG GGGTCCGCGG TGAGATAACT 720
GAGGCGACCC ATGCGATCTT GCAGCTCGCC GCTGCTCTGT ATGAGTTTGC GGCGGCCTGG 780
GGCCAGGCCC CTGCGCCCGCA GCTGCAAGGG GCTGGGGATGT AGCGATCAAC TGCCGCAATC 840
TTTCGCGCAA GGGGCGCCCG GTGCGACGCAA CCTGCTATCC GTACGGAAAT CGGGCCGGCA 900
TGCGTTGTCA AAAATGTGGT AATAAAGCCTC GAGCATGCTG CTGGTCTAGT ACACGATGAC 960
CAAGCTGACT AACCAGACCA ACCAACCTGGC TAATCTCAATG CTGAGCCGCA GCCAGATGAC 1020
CCAGGSGTAAT ATGGAATGGT TCGCAGGGG TGAGAACCAC GCACGTCTCG CCATCTCGGG 1080
CAACGGTCCTC GGGCGATGCA TGACTGGTCT CCTGCAATCC TCTCTCGGGG CAGCAGCGCTT 1140
GCAGGGCGTG AGCGCCGCGCC GTGCAATTCAA CAGGTGGGTT AAATGCTACT CAGTGGGGGT 1200
GGGCCAGAAT GCTGCGGCTGA GTGCGTTGAG TAACTCAGGC ACCAACGCTG AGCGTACCAA 1260
CCGCCACTTT GTGAGTAAAA AAGATCGCGG CATGCGAAAA GAGATCGGCC AGTGTATGGA 1320
TCAGTTACCG GAAATATCCC GTAAACCGGA ATACCAAGAAA GATGGCTGGG GTTGCGGAAA 1380
GACCGAGAC AAATCCTCGG GTAAGACGCT GAGTAAAAGC GATGATAGGC GTATGAGCGG 1440
CCGCCAGGATG GAACAAAACT GTCGACGCGT GGGGATAGAT CAAAAGCGGG TGCCGGGTGA 1500
TACC GGCAAT ACCACCATCC ACCTCGCTGG CCGCAGCGGTG GCATCGCTGG GTACGGATGC 1560
GGCTGCTGTC GGGCGATAAA TAGCAACATG TGCCGCTGGGT AAGCTGGCAA AGGCGATTGA 1620
ATCGGCTGCG GCCCTGAAAA GCGGAAACGA AAAAAGACAG GGGGAAGCCT GTCTGTTTTC 1680
TTATTGCGG GTTTATGCGG TTACCGGAC CGGTTAATCA TGCGTACGGA TCCTGGTACAA 1740
ACGCACATTT TCGGTCTCTAT CGCCTGCTGGT ACGGCCCAA ATCGCGATGC CACTCTCTTC 1800
GTGCGCTCAGA TTGGCGGGCT GTAGGGGAAC GGGCGGGTTGA ATATAGAAGAA ACTGCCGGGC 1860
The hypersensitive response elicitor polypeptide or protein derived from
*Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 23 as
follows:

```
   Met  Ser  Leu  Asn  Thr  Ser  Gly  Leu  Gly  Ala  Ser  Thr  Met  Gln  Ile  Ser
       1   5     10  15
     Ile  Gly  Gly  Ala  Gly  Gly  Asn  Asn  Gly  Leu  Leu  Gly  Thr  Ser  Arg  Gln
        20  25     30
    Asn  Ala  Gly  Leu  Gly  Gly  Asn  Ser  Ala  Leu  Gly  Leu  Gly  Gly  Gly  Asn
       35  40  45
   Gln  Asn  Asp  Thr  Val  Asn  Gln  Leu  Ala  Gly  Leu  Leu  Thr  Gly  Met  Met
        50  55  60
       65  70  75  80
       85  90  95
  Gly  Leu  Ser  Asn  Ala  Leu  Asn  Asp  Met  Leu  Gly  Gly  Ser  Leu  Asn  Thr
       100 105 110
Leu  Gly  Ser  Lys  Gly  Gly  Asn  Asn  Thr  Thr  Ser  Thr  Asn  Ser  Pro
       115 120 125
Leu  Asp  Gln  Ala  Leu  Gly  Ile  Asn  Ser  Thr  Ser  Gln  Asn  Asp  Asp  Ser
       130 135 140
  Thr  Ser  Gly  Thr  Asp  Ser  Ser  Asp  Ser  Ser  Asp  Pro  Met  Gln  Gln
       145 150 155 160
Leu  Leu  Lys  Met  Phe  Ser  Glu  Ile  Met  Gln  Ser  Leu  Phe  Gly  Asp  Gly
       165 170 175
  Gln  Asp  Gly  Thr  Gln  Gly  Ser  Ser  Gly  Gly  Lys  Gln  Pro  Thr  Glu
       180 185 190
  Gly  Glu  Gln  Asn  Ala  Tyr  Lys  Lys  Gly  Val  Thr  Asp  Ala  Leu  Ser  Gly
       195 200 205
```
This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from Erwinia amylovora is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, “Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora,” Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA
molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

```
AAGCTTCGGC ATGGCAGGT TGGACCTTTG GTCGGCAGGG TACGGTTGAA TTATTCATAA 60
GAGGAAATACG TTATGAGTCT GAATACAGAG GGGCTGGGAG GTCAACGAT GCACAATTTCT 120
ATGGCCGTTG CGGGCGGAAG TAACGGGTGT CGGGTATCAGA GTGCGCAGAA TGGCTGGTGTG 180
GGTTGCAATT CTGCACTGGG GCTGGCGGCG GTGAATCAGGA ATGATAAGCT CGAATGACTG 240
GCTGGCTTAC TCACGGCAT GTATGATGAT AGAAGACTGA TGGGGCGGTG TGGGCTAATG 300
GGCGGTTGGC TATGGCCTGC CGAATTTGAT GCTTTGCTGG GCTCAGGCTG CTCGGCGAA 360
GGACCTGTCA ACGGCGTGA GGTATGTTTA CGCGGTTCGG TGAACACGGT GGGCTGGCGA 420
GGCGGCAACA ATACACCTTC ACAAACAAAT TCCCCGCTGG ACCAGGCCTG GGGTATTAAC 480
TCACAGTCC AAAAAAAGCA CTCCCAGCTC GCCACAGATT CAACTCTCAG ATCAGTCGAC 540
CCGATGCAGC AGCTGTCTGA GATGTTTCACG GAGATAAGTGC AAAGCTCTTT TGGAATGGG 600
CAAGATGAGC CCCGCGGAG TCTCCTTCGG GCGAAGCAGC CGACGCGGAG CGACGCGAGGC 660
GGCTTATAAAA AAGGAGTACG TGGATCGCTG CGCGGCTCTA TAGGATATGG TCTGAGCCAG 720
CTCCTTGCCA AGGGCGGACT GGAAGGTGCT AAGGCTGGTA ATGCTGSCAC GGCTCTTGAC 780
GGTTGCTGCG TGGCGGCGAA AGGGCTGCAA AACCTGAGCG GCCCGGTGGA CTACAGCAGC 840
TTAGGTAACG CCGTGAGTAC CGGATCGGTG ATGAAACCGG GCATTCCAGG GCTGAAATGT 900
ATCGGTACGC ACAGGCGCCG TCTCAGCCTG TCTTTCGCTA ATAAAGGCGA TGGGGCGATG 960
GGCGAGGAAA TCGGGTCAGT CATGACCAAC TAGCTCAGAG CTGGGCGCAA GCGCGCTAGC 1020
CAGAAAGGCC CGGTGCGAGA GGTGAACCC CATGACCAAT CATGGAGCAA AGCAGCTAGC 1080
AAGCCAGATG AGCAAGCGAC AGATAGAGGC AGTCTGAAAC AAGCGCGGAGC 1140
ATGATCAAAG GGCGCATGGA GGGTGTACC GCAACCGCGAC ACCTGCACGC ACAGGGTGAC 1200
GGTGGTCTCT CGGCTGAACT ATGACCCATTG ATGGCCGCTGT ATGGCCATTAA CATATGGCA 1260
CTGGGCAAGC TGGGGCCGGC TTAAGCTT 1288
```

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 25 as follows:
| 1  | Met  | Gln  | Ser  | Leu  | Ser  | Leu  | Ser  | Ser  | Leu  | Gln  | Thr  | Pro  | Ala  | Met  |
| 10 | Ala  | Leu  | Val  | Leu  | Val  | Arg  | Pro  | Glu  | Ala  | Glu  | Thr  | Thr  | Gly  | Ser  | Thr  | Ser  | 30 |
| 35 | Ser  | Lys  | Ala  | Leu  | Gln  | Glu  | Val  | Val  | Val  | Lys  | Leu  | Ala  | Glu  | Glu  | Leu  | Met  | 45 |
| 55 | Arg  | Asn  | Gly  | Gln  | Leu  | Asp  | Ser  | Ser  | Asp  | Pro  | Leu  | Gly  | Lys  | Leu  | Leu  | Ala  | 60 |
| 70 | Lys  | Ser  | Met  | Ala  | Ala  | Asp  | Gly  | Lys  | Ala  | Gly  | Gly  | Ile  | Glu  | Asp  | Val  | 75 |
| 85 | Ile  | Ala  | Ala  | Leu  | Asp  | Lys  | Leu  | Ile  | His  | Glu  | Lys  | Leu  | Gly  | Asp  | Asn  | Phe  | 90 |
| 100| Gly  | Ala  | Ser  | Ala  | Asp  | Ser  | Ala  | Gly  | Ser  | Thr  | Gly  | Gln  | Gln  | Asp  | Leu  | Met  | 110|
| 115| Thr  | Gln  | Val  | Leu  | Asn  | Gly  | Leu  | Ala  | Lys  | Ser  | Met  | Leu  | Asp  | Leu  | Leu  | 120|
| 130| Thr  | Lys  | Gln  | Asp  | Gly  | Gly  | Thr  | Ser  | Phe  | Ser  | Glu  | Asp  | Asp  | Met  | Pro  | Met  | 140|
| 145| Leu  | Asn  | Lys  | Ile  | Ala  | Gln  | Phe  | Met  | Asp  | Asn  | Pro  | Ala  | Gln  | Phe  | Pro  | 160|
| 165| Lys  | Pro  | Asp  | Ser  | Gly  | Ser  | Trp  | Val  | Asn  | Glu  | Leu  | Lys  | Glu  | Asp  | Asn  | Phe  | 175|
| 180| Leu  | Asp  | Gly  | Asp  | Glu  | Thr  | Ala  | Ala  | Phe  | Arg  | Ser  | Ala  | Leu  | Asp  | Ile  | Ile  | 190|
| 195| Gly  | Gln  | Gln  | Leu  | Gly  | Asn  | Gln  | Gln  | Ser  | Asp  | Ala  | Gly  | Ser  | Leu  | Ala  | Gly  | 205|
| 210| Thr  | Gly  | Gly  | Leu  | Gly  | Thr  | Pro  | Ser  | Ser  | Phe  | Ser  | Asn  | Asn  | Ser  | Ser  | 220|
| 225| Val  | Met  | Gly  | Asp  | Pro  | Leu  | Ile  | Asp  | Ala  | Asn  | Thr  | Gly  | Pro  | Gly  | Asp  | Ser  | 240|
| 245| Gly  | Asn  | Thr  | Arg  | Gly  | Glu  | Ala  | Gly  | Gln  | Leu  | Ile  | Gly  | Glu  | Leu  | Ile  | Asp  | 255|
| 250| Arg  | Gly  | Leu  | Gln  | Ser  | Val  | Leu  | Ala  | Gly  | Gly  | Gly  | Leu  | Gly  | Leu  | Gly  | Thr  | Pro  | Val  | 270|
| 275| Asn  | Thr  | Pro  | Gln  | Thr  | Gly  | Thr  | Ser  | Ala  | Asn  | Gly  | Gly  | Gln  | Ser  | Ala  | Gln  | 285|
Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
290
295
300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305
310
315
320

5

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325
330
335

Asn Gln Ala Ala Ala
340

10

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, “*Pseudomonas syringae* pv. *syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 26 as follows:

20

ATGCAGAGTC TCAGTCTTAA CAGGAGCTCG CTGCAACACC CGGCAATGCC CTTTGTCTCG

GTACGTCTCTG AAGCCGAGAC GACTGGCAAGT AGGTGAGACA AGGCGCTTTCA GAAGTTTCTG

GTGAAAGCTGG CGGGGAAACT GATGGCCAAAT GGTCAACTCG AGCAGACGCTC GCCATTGGGA

AAACTGTGGG CCAAAGCTGAT GCCGCCAGAT GCCAAGGGCG GCCGGGCTTAT TGGAGATGTC

240

ATGCGCTGGGC TGGCAAGAGCT GATCCATGAA AAGCTCGGTT ACAACTTGCAG CGCGTCTGCG

GACGCCGCTT CGGATACGGG AGCCGAGAAC CTGATGACTG AGGTGCTCAA TGGCCTGGCC

AAATGGGAGC TGCTGAAACAAA GATGGGGCGG TCTATGGATG ACAATCCGGC AGCACTTCCC

540

AAACCGGACT CGGGCTCTCGG GTGTAAGGAA CTCAAGGAAG ACACTTTCGG TGATGGCGGC

25

30

GAAACGGCTGG GTTGGAGGTTG GGACCTGACG ATCATTGGCC AGCRACTGGG TAATCAGCAG

AGTGACGCTGG CGACTCTGGG AGGCGAGGT GGAGGGCTTG CGACGTGGG CAGTTTTGTCC

AAACACTGGGG CCGGATAGGGT TGATCCGGTG ATGACGCCCA ATACCGTCGC CGGTCAGAGC

GGCAATTACC CTTGGTGAAGC GGGGCACCTGG ATCGGCGAGC TTTATGGAGC TGCGCCCTCAG

TGGTATTGTC CGCGTGGGTTG ACTGGGCACA CCGTAAACA CCGCGGACG CGTACGCGG

320
330
340

60
120
180
240
300
360
420
480
540
600
660
720
780
840
The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 27 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
1     5     10    15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
20    25    30
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
35    40    45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
50    55    60
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
65    70    75    80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
85    90    95
Ala Asn Lys Thr Gly Asn Val Asp Ala Asn Gln Asp Pro Met  
100   105   110
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
115   120   125
Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
130   135   140
Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
145   150   155   160
Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
165   170   175
180   185   190
Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195   200   205
```
Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
210 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225 230 235 240
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
245 250 255
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gln Asn Gln
260 265 270
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
275 280 285
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
290 295 300
 Gln Gln Asn Asn Leu Gln Ser Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305 310 315 320
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
325 330 335
 Gln Ser Thr Ser Thr Gln Pro Met
340
It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.

ID. No. 28 as follows:

```
ATGTCAGTCC GAAACATCCA GAGCCCGTCC AACCCTCCGG GTCTGCAGAA CCGAACCTCT
  60
AAACACCAAC CCAACAGCCA GCAATCCGAC CAGTCCGTTGC AAGGACCTGAC CAGACCGGCTC
 120
GAGAAGGACA TCTCTAATCT CATCGCCAGCC CTCGTCAGACA AGGCGCGACA GTGCGCGGGC
 180
GGCAACCCGG GTAACCCCGG CAACCGCGCG GCGAAGGACG GCATGGCCAA CCGGCGGGCC
 240
AAGGACCGGA GCAAGAAGCA CGCGGACAGG AGCCAGAGCTC CCGAAGTCGCG AAACCAAGCC
 300
GGCAGAGTCG AGCAGCGCGA CAAACGGCAT CGGTGCAAAG CGCTGATGCA GTGCGTGGAA
 360
GACCTGGTGA AGCGTCTGAA GCGGGCTCTG CACATGACGC AGGCGCGGGC CAAATGCAAG
 420
GGCAACCGGC TGCGGGCTGC CAACGGGCGG AAGGATGGCG GCAGCCAGGGG GCGCCTGGCC
 480
GAAGGCGTGC AGAGAATCGA CGCATCTCTC GCCCAAGCTCG GCCCGGCGCG GCCGCGCGCG
 540
GGCGCGCGCG GTGGCGGGTG CGCGGTGGCT GTGGCGGGCG ATGGCGGGCTC CGTTGGCGGT
 600
GGCGAGCGGC GTGGCAAGCG CGCGASCAGG GCAGATGGCG TGAAAGCGCA CAGGGCGAAC
 660
GGCCGCAGCA AGCGAGGCGA TGCTACCGGT GCCAACCGGCG CGGATGACGG CAGGCGAACG
 720
CAGGGCGGCC TCACCGGGGT GCTGCAAAGA CTGATGAAGA TCTCTAACGC GCTGCGGCG
 780
```

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 29 as follows:

```
  Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
  1  5 10

20 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
  20 25
```

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 30 as follows:

```
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
  1  5 10 15

35 Leu Leu Ala Met
  20
```


The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.
Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed in vitro or in vivo in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or Staphylococcus proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of an Erwinia hypersensitive response elicitor which fragments elicit a hypersensitive response are fragments of the Erwinia amylovora hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 can span amino acids 105 and 403 of SEQ. ID. No. 23. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the
following amino acids of SEQ. ID. No. 23: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal
fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following
amino acids of SEQ. ID. No. 23: 76 and 209, 105 and 209, 99 and 209, 137 and 204,
137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. Other
suitable fragments can be identified in accordance with the present invention.

Variants may be made by, for example, the deletion or addition of amino acids
that have minimal influence on the properties, secondary structure and hydropathic
nature of the polypeptide. For example, a polypeptide may be conjugated to a signal
(or leader) sequence at the N-terminal end of the protein which co-translationally or
post-translationally directs transfer of the protein. The polypeptide may also be
conjugated to a linker or other sequence for ease of synthesis, purification, or
identification of the polypeptide.

The fragment of the present invention is preferably produced in
purified form (preferably at least about 60%, more preferably 80%, pure) by
conventional techniques. Typically, the fragment of the present invention is produced
but not secreted into the growth medium of recombinant host cells. Alternatively, the
protein or polypeptide of the present invention is secreted into growth medium. In the
case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., E. coli)
carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical
treatment, and the homogenate is centrifuged to remove bacterial debris. The
supernatant is then subjected to heat treatment and the fragment is separated by
centrifugation. The supernatant fraction containing the fragment is subjected to gel
filtration in an appropriately sized dextran or polyacrylamide column to separate the
fragment. If necessary, the protein fraction may be further purified by ion exchange
or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response
elicitor polypeptide or protein can be incorporated in cells using conventional
recombinant DNA technology. Generally, this involves inserting the DNA molecule
into an expression system to which the DNA molecule is heterologous (i.e. not
normally present). The heterologous DNA molecule is inserted into the expression
system or vector in proper sense orientation and correct reading frame. The vector
contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.IB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see “Stratagene Cloning Systems” Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., “Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,” Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccina virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression
elements of these vectors vary in their strength and specificities. Depending upon the
host-vector system utilized, any one of a number of suitable transcription and
translation elements can be used.

Different genetic signals and processing events control many levels of gene
expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is
a DNA sequence that directs the binding of RNA polymerase and thereby promotes
mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of
procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic
signals may not be recognized in or may not function in a procaryotic system, and,
further, procaryotic promoters are not recognized and do not function in eucaryotic
cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of
the proper procaryotic signals which differ from those of eucaryotes. Efficient
translation of mRNA in procaryotes requires a ribosome binding site called the Shine-
Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide
sequence of mRNA that is located before the start codon, usually AUG, which
encodes the amino-terminal methionine of the protein. The SD sequences are
complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably
promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct
positioning of the ribosome. For a review on maximizing gene expression, see
Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby
incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription).

For the purposes of expressing a cloned gene, it is desirable to use strong promoters in
order to obtain a high level of transcription and, hence, expression of the gene.
Depending upon the host cell system utilized, any one of a number of suitable
promoters may be used. For instance, when cloning in E. coli, its bacteriophages, or
plasmids, promoters such as the T7 phage promoter, lac promoter, trp promoter, recA
promoter, ribosomal RNA promoter, the Pr and PL promoters of coliphage lambda
and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be
used to direct high levels of transcription of adjacent DNA segments. Additionally, a
hybrid trp-lacUV5 (tac) promoter or other E. coli promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the lac operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as trp, pro, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in “strength” as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various “strong” transcription and/or translation initiation signals. For instance, efficient translation in E. coli requires an SD sequence about 7-9 bases 5' to the initiation codon (“ATG”) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the cro gene or the N gene of coliphage lambda, or from the E. coli tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment itself elicits a hypersensitive response; in a
non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment elicits a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment elicits a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a genes encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment elicits a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seeds cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.
The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, inter alia, to the following viruses can be achieved by the method of the present invention: Tobacco mosaic virus and Tomato mosaic virus. Resistance, inter alia, to the following bacteria can also be imparted to plants in accordance with present invention: Pseudomonas solancearum, Pseudomonas syringae pv. tabaci, and Xanthomonas campestris pv. pelargonii. Plants can be made resistant, inter alia, to the following fungi by use of the method of the present invention: Fusarium oxysporum and Phytophthora infestans.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased
quantity of seeds produced, increased percentage of seeds germinated, increased plant
size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit
and plant maturation. As a result, the present invention provides significant economic
benefit to growers. For example, early germination and early maturation permit crops
to be grown in areas where short growing seasons would otherwise preclude their
growth in that locale. Increased percentage of seed germination results in improved
crop stands and more efficient seed use. Greater yield, increased size, and enhanced
biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of
insect control for plants. For example, insect control according to the present
invention encompasses preventing insects from contacting plants to which the
hypersensitive response elicitor has been applied, preventing direct insect damage to
plants by feeding injury, causing insects to depart from such plants, killing insects
proximate to such plants, interfering with insect larval feeding on such plants,
preventing insects from colonizing host plants, preventing colonizing insects from
releasing phytotoxins, etc. The present invention also prevents subsequent disease
damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European
corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200
plant species including green, wax, and lima beans and edible soybeans, peppers,
potato, and tomato plus many weed species. Additional insect larval feeding pests
which damage a wide variety of vegetable crops include the following: beet
armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth,
cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm),
pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect
pests represents the most economically important group of pests for vegetable
production worldwide.

The method of the present invention involving application of the fragment of a
hypersensitive response elicitor polypeptide or protein, which fragment elicits a
hypersensitive response, can be carried out through a variety of procedures when all
or part of the plant is treated, including leaves, stems, roots, etc. This may (but need
not) involve infiltration of the hypersensitive response elicitor polypeptide or protein
into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment elicits a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include \((\text{NH}_4\text{)}_2\text{NO}_3\). An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can
be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response eliciting fragment need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.


Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaces bodies. Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., *Proc. Natl. Acad. Sci. USA*, 82:5824 (1985), which is
hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with _Agrobacterium tumefaciens_ or _A. rhizogenes_ previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

_Agrobacterium_ is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (_A. tumefaciens_) and hairy root disease (_A. rhizogenes_). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of _A. tumefaciens_ or the Ri plasmid of _A. rhizogenes_. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, _Science_, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response eliciting fragment resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response eliciting fragment is applied. These other materials, including hypersensitive response eliciting fragments,
can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response eliciting fragment to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

**EXAMPLES**

**Example 1 - Strains and plasmids used**

The strains and plasmids used are set forth in Table 1 below.

<table>
<thead>
<tr>
<th>Plasmid name</th>
<th>E. amylovora source strain</th>
<th>Brief Description, Relevant Phenotype, Reference</th>
<th>Harpin, fragment (or NA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBCKS</td>
<td>-</td>
<td>Cm' derivative of pBluescript KS, Stratagene, La Jolla, CA</td>
<td></td>
</tr>
<tr>
<td>pBCSK</td>
<td>-</td>
<td>Cm' derivative of pBluescript SK, Stratagene, La Jolla, CA</td>
<td></td>
</tr>
<tr>
<td>pBSKS</td>
<td>-</td>
<td>pBluescript KS; Ap', Stratagene, La Jolla, CA</td>
<td></td>
</tr>
<tr>
<td>pBSKK II</td>
<td>-</td>
<td>pBluescript II SK; Ap', Stratagene, La Jolla, CA</td>
<td></td>
</tr>
<tr>
<td>pBW7</td>
<td>-</td>
<td>Mob+Tc' helper plasmid. (Rella et al., &quot;Transposon Insertion Mutagenesis of <em>Pseudomonas aeruginosa</em> With a Tn5 Derivative: Application to Physical Mapping of the <em>arc</em> Gene Cluster,&quot; <em>Gene</em> 33:293-303 (1983), which is hereby incorporated by reference)</td>
<td>NA</td>
</tr>
<tr>
<td>pCPP51</td>
<td>-</td>
<td>Broad host range derivative of pBSKK II containing ori from pRO1614</td>
<td>NA</td>
</tr>
<tr>
<td>pCPP460</td>
<td>246</td>
<td>Functional <em>hrp</em> gene cluster of <em>E. amylovora</em> Ea246 cloned into pCPP9</td>
<td></td>
</tr>
<tr>
<td>pCPP1104</td>
<td>321</td>
<td>1.2 kb <em>PstI</em> fragment of pCPP1084 in pBCKS</td>
<td>Ea C31</td>
</tr>
<tr>
<td>pCPP1105</td>
<td>321</td>
<td>1.1 kb <em>SmaI</em> fragment of pCPP1084 in pBCKS</td>
<td>Ea C82</td>
</tr>
<tr>
<td>pCPP1107</td>
<td>321</td>
<td>1.3 kb <em>HindIII</em> fragment of pCPP1084 in pBCKS</td>
<td>Ea wt</td>
</tr>
<tr>
<td>pCPP1108</td>
<td>321</td>
<td>1.2 kb <em>HindII-HindIII</em> of pCPP1084 in pBCKS</td>
<td>Ea N11</td>
</tr>
<tr>
<td>pCPP1109</td>
<td>321</td>
<td>pCPP1107 with internal <em>Avall</em> fragment deleted</td>
<td>Ea 1175</td>
</tr>
<tr>
<td>pCPP1110</td>
<td>321</td>
<td>As pCPP1108, but cloned into pBCSK</td>
<td>Ea N9</td>
</tr>
<tr>
<td>pCPP1111</td>
<td>321</td>
<td>367 bp <em>TaqI</em> fragment of pCPP1107 in pBCKS</td>
<td>Ea C305</td>
</tr>
<tr>
<td>pCPP1113</td>
<td>246</td>
<td>As pCPP1109, but 425bp Avall fragment of pCPP1098 deleted</td>
<td>Ear 1175</td>
</tr>
<tr>
<td>pCPP1119</td>
<td>246</td>
<td>Site specific mutation in pCPP1098; stop codon inserted at L36</td>
<td>Ear C368</td>
</tr>
<tr>
<td>pCPP1120</td>
<td>246</td>
<td>Site specific mutation in pCPP1098; stop codon inserted at T123</td>
<td>Ear C281</td>
</tr>
<tr>
<td>pCPP1121</td>
<td>321</td>
<td>702bp KpnI fragment internal to hrpN deleted</td>
<td>Ea C375</td>
</tr>
<tr>
<td>pCPP1127</td>
<td>246</td>
<td>3.1 kb BamHI fragment of pCPP1098 in pSU21</td>
<td>Ear wt</td>
</tr>
<tr>
<td>pCPP1128</td>
<td>246</td>
<td>Tn10 mini kan in pCPP1127</td>
<td>Ear undef</td>
</tr>
<tr>
<td>pCPP1136</td>
<td>246</td>
<td>4.4 kb EcoRI fragment of pCPP1120, religated</td>
<td>EAR N122</td>
</tr>
<tr>
<td>pCPP1146</td>
<td>246</td>
<td>4.2 kb EcoRI fragment of pCPP1119, religated</td>
<td>Ear N35</td>
</tr>
<tr>
<td>pCPP1147</td>
<td>321</td>
<td>1.2 kb BamHI fragment of pCPP1084, PCR amplified, cloned into pSU23</td>
<td>Ea wt</td>
</tr>
<tr>
<td>pCPP1148</td>
<td>246</td>
<td>As pCPP1147, but from pCPP1098</td>
<td>Ear wt</td>
</tr>
<tr>
<td>pCPP1150</td>
<td>246</td>
<td>As pCPP1148, but in pCPP51 vector</td>
<td>Ear wt</td>
</tr>
<tr>
<td>pCPP1163</td>
<td>246</td>
<td>3.1 kb BamHI fragment of pCPP1098 in pCPP51</td>
<td>Ear wt</td>
</tr>
<tr>
<td>pCPP1164</td>
<td>321</td>
<td>1.3 kb HindIII of pCPP1084 in pCPP51</td>
<td>Ea wt</td>
</tr>
<tr>
<td>pCPP1165</td>
<td>321</td>
<td>Derivative of pCPP51 w/ KpnI, SacII sites removed</td>
<td>NA</td>
</tr>
<tr>
<td>pCPP1167</td>
<td>321</td>
<td>1.3 kb HindIII fragment of pCPP1107 in pCPP1165</td>
<td>Ea wt</td>
</tr>
<tr>
<td>pCPP1169</td>
<td>246</td>
<td>As pCPP1167, but 3.1 kb BamHI insert from pCPP1098</td>
<td>Ear wt</td>
</tr>
<tr>
<td>pCPP1170</td>
<td>246</td>
<td>pCPP1098; Sigma-Sp ligated into EcoRV site</td>
<td>Ear C1285</td>
</tr>
<tr>
<td>pCPP1171</td>
<td>246</td>
<td>KpnI fragment internal to hrpN deleted; shifted reading frame</td>
<td>Ea C375</td>
</tr>
<tr>
<td>pCPP1172</td>
<td>321</td>
<td>Derivative of pCPP1167 with in-frame deletion of KpnI fragment internal to hrpN</td>
<td>Ea I235</td>
</tr>
<tr>
<td>pCPP1173</td>
<td>246</td>
<td>As pCPP1172, but from pCPP1169</td>
<td>Ea I235</td>
</tr>
<tr>
<td>pCPP217</td>
<td>321</td>
<td>pCPP1084 with 2 StyI fragments deleted, blunt ended, and religated</td>
<td>Ea C185</td>
</tr>
<tr>
<td>pCPP1252</td>
<td>321</td>
<td>pCPP1103 with Sigma-Sp ligated at Smal site</td>
<td>Ea C825</td>
</tr>
<tr>
<td>pH455</td>
<td></td>
<td>Ap'; Sp'; source of Omega-Sp fragment; (Fellay, R., et al., &quot;Interposon Mutagenesis of Soil and Water Bacteria a Family of DNA Fragments Designed for in vitro Insertional Mutagenesis of Gram-Negative Bacteria,&quot; Gene, 52:147-154 (1987), which is hereby incorporated by reference).</td>
<td>N/A</td>
</tr>
<tr>
<td>Omega</td>
<td></td>
<td>P15a ori Km' (Bartolomé, B.Y., et al., &quot;Construction and properties of a Family of pACYC184-Derived Cloning Vectors Compatible With pBR322 and its Derivatives,&quot; Gene, 102:75-78 (1991), which is hereby incorporated by reference).</td>
<td>N/A</td>
</tr>
<tr>
<td>PSU23</td>
<td></td>
<td>P15a ori Km' (Bartolomé, B.Y., et al., &quot;Construction and properties of a Family of pACYC184-Derived Cloning Vectors Compatible With pBR322 and its Derivatives,&quot; Gene, 102:75-78 (1991), which is hereby incorporated by reference).</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Strains used**

*E. amylovora*  
Ea273Nx; Nalidixic acid resistant (Nx') derivative of Ea273. CUCPB 2348

*E. amylovora*  
Rifampin resistant derivative of Ea32. CUCPB 2545
**E. coli**  | GM272; *dam-, dcm-*; CUCPB 3047; (Blumenthal, R.M., et al., “E. coli Can Restrict Methylated DNA and May Skew Genomic Libraries,” Trends in Biotech, 4:302-305 (1986), which is hereby incorporated by reference)

**E. coli**  | BL21(DE3); CUCPB 4277; (Studier, F.W., and B.A. Moffatt, “Use of Bacteriophage T7 RNA Polymerase to Direct Selective High-level Expression of Cloned Genes,” J. Mol. Biol., 189:113-130 (1986), which is hereby incorporated by reference)

**E. coli**  | DH5α; (N<sup>+</sup>). CUCPB 2475; Stratagene, La Jolla, CA.

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**Example 2 - Molecular biology techniques.**

Several approaches were employed to obtain truncated or otherwise altered versions of both *E. amylovora* harpins. These techniques included: (i) subcloning of restriction fragments containing portions of the gene encoding the hypersensitive response elicitor protein or polypeptide from *Erwinia amylovora* (i.e. *hrpN*) into expression vectors, by standard techniques (Sambrook, et al., Molecular Cloning: a Laboratory Manual, 2<sup>nd</sup> ed. ed. Cold Spring Harbor, Laboratory,” Cold Spring Harbor, NY (1989), which is hereby incorporated by reference); (ii) insertion of an Ω-fragment (Fellay, et al., “Interposon Mutagenesis of Soil and Water Bacteria a Family of DNA Fragments Designed for in vitro Insertional Mutagenesis of Gram-Negative Bacteria,” Gene 52:147-154 (1987), which is hereby incorporated by reference) into *hrpN*; (iii) site-specific mutagenesis approaches (Innis, et al., PCR Protocols. A Guide to Methods and Applications, Academic Press San Diego, CA (1990); Kunkel, et al., “Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection,” Proc. Nat. Acad. Sci. USA 82:488-492 (1985), which are hereby incorporated by reference); and (iv) creation of nested deletions (Erase-a-Base<sup>TM</sup> kit; Promega, Madison, WI). C-terminal deletion analysis of the hypersensitive response elicitor protein or polypeptide from *Erwinia amylovora* (i.e. harpin<sub>Ea</sub>) in pCPP1084 could not be performed because of the location of restriction enzyme cleavage sites in pCPP1084. For N-terminal deletions, pCPP1084 DNA was prepared using a Qiagen midiprep column (Qiagen, Chatsworth, CA) and digested with *sst I* followed by *EcoRI*. Subsequently, the digested DNA was subjected to exonuclease III digestion, ligation, and transformation into *E. coli* BL21(DE3). Deletion sizes were estimated by agarose gel electrophoresis. Harpin fragments were named with respect to the portion of harpin deleted (e.g., harpin<sub>Ea</sub> C82 lacks the C-terminal 82 amino acid residues of full-length harpin<sub>Ea</sub>).
Example 3 - Protein expression.

For expression from T7 promoters, T7 RNA polymerase-dependent systems were used. These systems utilized either strain *E. coli* BL21(DE3) (Studier, et al., “Use of Bacteriophage T7 RNA Polymerase to Direct Selective High-Level Expression of Cloned Gene,” *J. Mol. Biol.* 189:113-130 (1986), which is hereby incorporated by reference), or plasmid pGPI-2 (Tabor, et al., “A Bacteriophage T7 DNA Polymerase/Promoter System for Controlled Exclusive Expression of Specific Genes,” *Proc. Natl. Acad. Sci.*, USA 82:1074-1078 (1985), which is hereby incorporated by reference) in *E. coli* DH5α. Expression of *hrpN* from the T7 promoter was induced by addition of IPTG to a final concentration of 0.4 mM. For expression in *E. amylovora* Ea321 (i.e. harpinaa) or Ea273, pGPI-2 was introduced by transformation with a 42°C heat shock for 10 minutes, or by electroporation (Biorad Gene Pulser™). Hypersensitive response (i.e. HR)-eliciting activity was screened in tobacco cv. Xanthi leaves by in planta lysis (He, et al., “*Pseudomonas syringae* pv. *syringae* harpinaa: a Protein That is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference) or by preparation of boiled and unboiled “CFEPs” (Wei, et al., “Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,” *Science* 257:85-88 (1992), which is hereby incorporated by reference).

Example 4 - *In vitro* proteolysis of harpin.

*In vitro* proteolysis of harpinaa with *Staphylococcus* V8 proteinase (also termed endoproteinase Glu-C), trypsin, pepsin, and papain was performed as recommended (Scopes, et al., *Protein Purification: Principles and Practice*, 2nd ed. Springer-Verlag. New York (1987), which is hereby incorporated by reference), for 2-16 hrs. at 20-37°C. Endoproteinase Glu-C digestion was performed either in 50 mM ammonium bicarbonate, pH 7.8 (in which cleavage occurs only after glutamic acid), or in 50 mM potassium phosphate, pH 7.8 (in which cleavage after both glutamic acid and aspartic acid occurs).
Example 5 - Plant-derived proteinases.

Intercellular fluids (IF) were obtained from tobacco, tomato, apple, raspberry, and cotoneaster, as described (Hammond-Kosack, et al., "Preparation and Analysis of Intercellular Fluid," p. 15-21. In S.J. Gurr, M.J. McPherson, and D.J. Bowles (ed.), Molecular Plant Pathology A Practical Approach, 2nd ed., The Practical Approach Series, IRL Publishers, Oxford (1992), which is hereby incorporated by reference), by vacuum infiltration of intercellular spaces with high-purity water. Proteolytic digestion of PAGE-purified harpin$_{Ea}$ was performed for 2-16 hrs. at 20-37°C, pH by mixing equal volumes of IF with harpin$_{Ea}$. A total leaf extract was obtained by grinding tobacco leaf panels with mortar and pestle in 5 mM potassium phosphate. The extract was centrifuged and filtered, and the clarified ground leaf extract used identically as was the IF. Proteinase inhibitors were employed as follows: Pepstatin A (final concentration 1μM), E-64 (1μM), Aprotinin (2μg/ml), o-phenanthroline (1mM), and p-mercuribenzoate (PCMB) (Sigma, St. Louis, MO).

Example 6 - Peptide purification.

Peptide fragments of harpin obtained following digestion with tobacco IF were fractionated by reverse-phase HPLC on a Vydae C18 column using a 2-60% acetonitrile gradient in 0.1% trifluoroacetic acid. Fractions were lyophilized, resuspended in 5 mM potassium phosphate and infiltrated into tobacco leaf panels. The fraction with greatest HR-eliciting activity was refractionated as above with a 35-70% acetonitrile gradient, and the purity of each fraction was assayed via gas chromatography-mass spectroscopy (GC-MS) and by N-terminal protein sequencing at the Cornell Biotechnology Program Core Facility.

Example 7 - Proteinase activity-stained gels.

Proteinase activity of IF was assayed in activity-stained polyacrylamide gels (Laemmli, “Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4,” Nature 227:680-685 (1970), which is hereby incorporated by reference) copolymerized with 0.1% gelatin (Heussen, et al., “Electrophoretic Analysis of Plasminogen Activators in Polyacrylamide Gels Containing Sodium
Dodecyl Sulfate and Copolymerized Substrates,” Anal. Biochem. 102:196-202 (1980), which is hereby incorporated by reference). After electrophoresis, each gel was rinsed extensively to remove SDS and allow refolding of proteinases in the gel. Following additional incubation to allow proteolysis to occur, the gels were stained with 0.1% Amido Black in 30% methanol/10% acetic acid. Each gel stained darkly (due to the presence of copolymerized gelatin) except where proteinases had digested the gelatin, resulting in colorless bands representing the sites of proteinase activity.

**Example 8 - Truncated harpins retain HR-eliciting activity.**

The stability and the HR-eliciting activity of proteins encoded by various DNA constructs is shown in Figure 1. Many DNA constructs encoding portions of harpin\textsubscript{Ea} or harpin\textsubscript{Ea} did not yield detectable protein products following induction of expression in the T7 promoter-polymerase system (Tabor, et al., “A Bacteriophage T7 DNA Polymerase/Promoter System for Controlled Exclusive Expression of Specific Genes,” Proc. Natl. Acad. Sci. USA 82:1074-1078 (1985), which is hereby incorporated by reference) and analysis of cell extracts by PAGE, possibly due to instability of the encoded proteins. No DNA constructs (e.g., those obtained via Erase-a-Base\textsuperscript{TM} protocol) yielded detectable protein products displaying N-terminal deletions relative to the full-length protein. No stable but inactive proteins were identified. Several constructs encoding proteins truncated at their C-terminus and often including additional vector-encoded amino acids yielded detectable products (e.g. harpin\textsubscript{Ea} C82). In contrast, a construct encoding the same 321 N-terminal amino acid residues of harpin\textsubscript{Ea}, but yielding a protein truncated by the presence of an Ω-fragment (harpin\textsubscript{Ea} C82 Ω) was unstable (i.e. no product was detected). A construct encoding a harpin\textsubscript{Ea} fragment with a large internal deletion (harpin\textsubscript{Ea} I175) was also successfully used to express protein. These various truncated proteins were tested for HR-eliciting activity. A 98 residue N-terminal harpin\textsubscript{Ea} fragment (harpin\textsubscript{Ea} C305) was the smallest bacterially-produced peptide that displayed HR-eliciting activity.
Example 9 - Secretion of harpin<sub>Ea</sub> with an altered C-terminus.

The effect of alteration at the harpin C-terminus on its secretion was examined. Harpin C31 contains the N-terminal 372 amino acids of harpin, but lacks the C-terminal 31 residues, which are replaced by 47 residues encoded by the vector, resulting in a protein slightly larger than the wild type harpin<sub>Ea</sub>. The C31 protein retains HR-eliciting activity and is stable and easily expressed and detected by western analysis or PAGE but it is no longer secreted into the culture supernatant as is the wild type protein (Figure 2). The presence of harpin<sub>Ea</sub>C31 does not interfere with secretion of the wild type harpin, which is found in both the CFEP and the culture supernatant. However, harpin<sub>Ea</sub>C31 is found only in the CFEP.

Example 10 - Effect of proteolysis on harpin<sub>Ea</sub>’s HR eliciting activity

In order to generate additional harpin<sub>Ea</sub> fragments, purified full length protein was proteolyzed in vitro by several proteinases, including endoproteinase Glu-C, trypsin, pepsin, and papain (e.g., Figures 3 and 4). Harpin solutions digested with trypsin or with papain lost all activity. In contrast, following digestion with endoproteinase Glu-C, HR-eliciting activity was retained. No peptides larger than 6 kD were evident by PAGE following trypsin digestion. Endoproteinase Glu-C digestion yielded an approximately 20 kD fragment, larger than expected if all cleavage sites were cut, indicating that digestion was not complete (Figure 4).

Example 11 - Apoplastic fluids (IF) contain harpin-degrading proteolytic activity

Apoplastic fluids (intercellular fluids; IF) from tobacco and other plants were also employed to proteolyze harpin. Each IF tested possessed proteinase activity(s), as indicated by the presence of multiple activity-stained bands in polyacrylamide gels containing co-polymerized gelatin (Figures 5A to 5C), as well as by the disappearance of detectable harpin<sub>Ea</sub> (Schägger, et al., “Tricine-Sodium Dodecyl Sulfate Gel Electrophoresis for the Separation of Proteins in the Range From 1 to 100 kDa,” Anal. Biochem. 166:368-379 (1987), which is hereby incorporated by reference) following overnight digestion of purified harpin<sub>Ea</sub> with IF. Proteinase activity was substantially greater at 37°C than at 20°C, and activity was higher at pH 8.5 than at pH 7. Several
inhibitors were employed in order to define the proteolytic activity(s) of the IF. No single proteinase inhibitor which was employed prevented degradation of harpin$_{Ea}$. However, a mixture of the inhibitors Pepstatin A (1µM), E-64 (1µM), Aprotinin (2µg/ml), and o-phenanthroline (1mM), targeted at acid proteinases, cysteine proteinases, serine proteinases, and metalloproteinases, respectively, partially inhibited proteolysis.

Harpin$_{Ea}$ degraded by proteolytic activities present in the plant apoplast retained HR-eliciting activity (Figure 3). In contrast, harpin$_{Ea}$ proteolyzed by a clarified extract produced by grinding tobacco leaf tissue with mortar and pestle lost HR-eliciting activity. In order to study whether apoplastic degradation of harpin was a prerequisite to its HR-eliciting activity, the length of time required for leaf collapse when either intact harpin or harpin predigested with tobacco IF was infiltrated into tobacco leaf panels was compared. Both preparations elicited the HR in a similar time frame (12-18 hours, depending on the experiment).

Example 12 - Characterization of HR-eliciting peptide fragments

Peptides resulting from digestion by apoplastic plant proteinase(s) were fractionated by reverse phase HPLC (Vydac C18 column), and tested for activity. Following treatment of intact harpin$_{Ea}$ with tobacco IF, three fractions contained some HR-eliciting activity on tobacco. Two of the three demonstrated weak activity, and little protein was present. They were not further characterized. Fraction 19, which contained the strongest activity as well as the most protein, was refractionated using a more shallow elution gradient (Figure 6). Refractionation, N-terminal protein sequencing, and molecular weight analysis by mass spectroscopy indicated that four largely overlapping peptides were present. Peak 19-1 contained peptides P91 and P95, corresponding to harpin$_{Ea}$ residues 110-200 and 110-204; peak 19-2 contained peptides P64 and P68, corresponding to harpin$_{Ea}$ residues 137-200 and 137-204. 19-1 and 19-2 each possessed HR-eliciting activity. The smallest peptide thus confirmed to retain activity consisted of residues 137-204. The two peptides in each peak were not separable under the conditions used. These active fragments are distinct from the smallest active N-terminal fragment (harpin$_{Ea}$C305), and indicate that more than one portion of harpin$_{Ea}$ displays activity in planta. Further digestion with trypsin
abolished the HR-eliciting activity of 19-2. This proteinase cleaves P64 and P68 as shown in Figure 7. Further digestion with endoproteinase Glu-C in ammonium bicarbonate buffer abolished the HR-eliciting activity of 19-1. Endoproteinase Glu-C is predicted to cleave P91 and P95 as shown in Figure 7. Loss of elicitor-activity followed further digestion of these peptides with endoproteinase Glu-C or trypsin.

**Example 13 - *E. amylovora* harpin's similarity with other proteins.**

The predicted protein sequences of proteinaceous HR elicitors from several other bacterial plant pathogens, and of other proteins known to be, or thought to be, secreted by a type III secretion pathway were also compared with that of harpin_{Ea}. When harpin_{Ea} was compared with elicitors from *E. amylovora* Ea246 (i.e. harpin_{Ea}), *Erwinia chrysanthemi* EC16 (harpin_{Ec}) (Bauer, et al., "Erwinia chrysanthemi harpin_{Ec}": An Elicitor of the Hypersensitive Response That Contributes to Soft-Rot Pathogenesis," *Mol. Plant-Microbe Interact* 8:484-491 (1995), which is hereby incorporated by reference), *Erwinia carotovora* subsp. *carotovora* (harpin_{Ec}) (Mukherjee, et al., Presented at the 8th International Congress Molecular Plant-Microbe Interactions, Knoxville, TN (1996), which is hereby incorporated by reference), *Erwinia stewartii* (Harpin_{Es}) (Frederick, et al., "The wts Water-Soaking

the *Erwinia*-derived harpins contained significant regions of similarity at the C-terminus. In addition, all the elicitors are glycine-rich, secreted, and heat-stable. Limited similarity between harpin\textsubscript{Ps} and harpin\textsubscript{Es} had been reported previously (He, et al., "*Pseudomonas syringae* pv. *syringae* harpin\textsubscript{Ps}: a Protein That is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference), (Laby, et al., Presented at the Seventh International Symposium on Molecular Plant-Microbe Interactions, Edinburgh, Scotland (1994), which is hereby incorporated by reference). A limited region of similarity between harpin\textsubscript{Es} and other harpins from *Erwinia* spp. was also evident at the extreme N-terminus of each protein, where 9 out of the first 26 residues are conserved (Figure 8). Kyte-Doolittle hydropathy plots for each of the harpins produced by different *Erwinia* spp. are illustrated in Figure 9. Each of the *Erwinia* harpins examined displays a generally similar hydrophobic profile along the full length of the protein. This profile is distinct from the profile demonstrated by PopA1 and by harpin\textsubscript{Ps}, and does not possess the symmetry evident in the profile of those two proteins. The hydropathy profile of each *Erwinia* harpin is generally similar to that of the others, yet distinct from that reported for harpin\textsubscript{Ps} (Alfano, et al., "Analysis of the Role of the *Pseudomonas Syringae* HrpZ harpin in Elicitation of the Hypersensitive Response to Tobacco Using Functionally Nonpolar *hrpZ* Deletion Mutations, Truncated HrpZ Fragments, and *hrmA* Mutations," *Mol. Microbiol.* 19:715-728 (1996), which is hereby incorporated by reference). Harpin\textsubscript{Ec} possesses a strikingly hydrophobic region around residues 54-143 (Mukherjee, et al., Presented at the 8\textsuperscript{th} International Congress Molecular Plant-Microbe Interactions, Knoxville, TN (1996), which is hereby incorporated by reference). This portion of the protein is also the most hydrophobic region of harpin\textsubscript{Es} and harpin\textsubscript{Es}. The rest of each protein is predominantly hydrophilic.

Truncated proteins and fragments of harpin obtained following proteolytic digestion of the full length protein indicate several surprising aspects of harpin\textsubscript{Es} HR-eliciting activity. These harpin fragments demonstrate that HR-eliciting activity resides in distinct regions of the protein, and that relatively small fragments of the protein, as little as 68 residues and possibly less, are sufficient for this activity. Fragments of other plant pathogen-derived elicitor proteins also retain biological

Expression of truncated harpin fragments and proteolysis of full-length harpins showed that two distinct fragments retain HR-eliciting activity. The primary sequence of each active fragment show no discernable similarity with each other, or with other elicitor-active peptides. However, the sites of cleavage by trypsin and endoproteinase Glu-C suggest portions of each fragment required for activity. It would be of interest to alter specifically the amino acid residues at or near these cleavage sites to determine whether HR-eliciting activity is altered or lost. Additionally, harpin_{Ea} P64 and P68 demonstrate distinct hydrophobicity during reverse-phase HPLC (Figure 6), and they correspond to a hydrophobic peak in a Kyte-Doolittle plot (Figure 9). The role of this putative hydrophobic domain could be tested by mutagenesis, or by synthesis of altered peptides. However, the fact that multiple fragments independently possess HR-eliciting activity complicates analysis of full-length proteins.

This finding, that fragments of the protein retain HR-eliciting activity, also allowed (at least) two apoplastic proteinase activities, which are distinct from intracellular plant proteinases, to be defined. Two apoplastic plant proteinases (from soybean) have been studied in some detail. SMEP, a metalloproteinase (Huangpu, et al., “Purification and Developmental Analysis of an Extracellular Proteinase From Young Leaves of Soybean,” *Plant Physiol.* 108:969-974 (1995); McGeehan, et al., “Sequencing and Characterization of the Soybean Leaf Metalloproteinase,” *Plant Physiol.* 99:1179-1183 (1992), which are hereby incorporated by reference) sensitive
to EDTA, is thought to cleave at G/L and G/I. Interestingly, although there are 19 potential SMEP cleavage sites in the intact harpin$_{Ea}$, only one of them is located within fragments P91 and P95, and none are within fragments P64 and P68 (Figure 7). P91 and P95 thus may represent partial digestion products of a SMEP-like proteinase in the tobacco apoplast. The other studies soybean apoplastic proteinase, SLAP, a sulfhydryl proteinase (Huangpu, et al., “Purification and Developmental Analysis of an Extracellular Proteinase From Young Leaves of Soybean,” Plant Physiol. 108:969-974 (1995), which is hereby incorporated by reference) sensitive to p-chloromercuribenzoic acid (pCMB). Several lines of evidence suggest that multiple proteolytic activities in the IF are degrading harpin$_{Ea}$. PMSF, a serine protease inhibitor, decreases but does not entirely block harpin$_{Ea}$ degradation (Figure 5C); no single proteinase inhibitor tested blocks harpin degradation, and the cleavage sites after residues 109, 136, 200, and 204 are dissimilar. Endoproteinase Glu-C does not abolish activity of full-length harpin, but does abolish activity of P91 and P95 (and presumably P64 and P68); trypsin abolishes the activity of P64 and P68 (and presumably P91 and P95). These final digests suggest specific portions of each distinct HR-eliciting peptide which are presumably necessary for its activity, as mentioned previously.

The apoplastic activities degrade harpin without destroying its HR-eliciting ability, in contrast to intracellular proteolytic activities present in a ground leaf-extract, which abolish activity. This raises a number of intriguing questions, e.g., does the plant use these harpin fragments as elicitor-signals? The timing of the HR was examined when full length harpin and harpin predigested by tobacco intercellular fluid were each infiltrated into tobacco leaves. The HR elicited by each preparation occurred 12-18 hours after infiltration. Co-infiltration of proteinase inhibitors into tobacco leaf panels along with harpin also had no effect on harpin’s HR eliciting activity, although limited proteolytic degradation cannot be ruled out in this case, particularly since it seems that at least two, and perhaps more, apoplastic proteinases are present in tobacco. Because predigested harpin elicited the HR no faster than undigested protein, proteolytic digestion seems unlikely to be a rate-limiting step required for HR to occur. The role of these apoplastic proteinases which are able to hydrolyze harpin partially, yet unable to abolish harpin’s HR-eliciting activity on
tobacco, remains unclear. Salzer et al., “Rapid Reactions of Spruce Cells to Elicitors Released From the Ectomycorrhizal Fungus *Hebeloma crustuliniforme* and Inactivation of These Elicitors by Extracellular Spruce Cell Enzymes,” *Planta* 198:118-126 (1996), which is hereby incorporated by reference, have noted that spruce (*Picea abies* (L.) Karst.) modulates the level of fungal cell wall elicitors released by the ectomycorrhizal fungus *Hebeloma crustuliniforme* by inactivating these molecules in the apoplast. They propose that *Picea* controls the elicitor level as part of its symbiotic interaction with the fungus. Similarly, PGIP of *Phaseolus vulgaris* has been suggested to modulate the level of elicitor-active oligogalacturonides present during the plant-parasite interaction in bean (Desiderio, et al., “Polygalacturonase, PGIP, and Oligogalacturonides in Cell-Cell Communication,” *Biochem. Sci. Trans.* 22:394-397 (1994), which is hereby incorporated by reference).

Perhaps the retention of HR-eliciting activity by harpin fragments contributes to the ability of plants to recognize the presence of a pathogen. In this regard, it would be interesting to explore whether transgenic host and non-host plants, engineered for apoplastic expression of a harpin activity-degrading proteinase, would exhibit reduced or increased sensitivity to *E. amylovora*, compared to non-engineered plants.

Despite numerous attempts, only a handful of truncated derivatives of harpin$_{Ea}$ and harpin$_{Ea}$ were successfully expressed from portions of *hrpN*. Problems with protein stability were evident in that several truncated harpins were unstable and difficult to purify. Additionally, expression of truncated harpins may be deleterious to bacteria. Truncated harpin$_{Ea}$C31 was, however, stable and easily purified, but not secreted, suggesting that C-terminal sequences are involved in harpin secretion. Unfortunately, the presence of vector-encoded amino acids in this protein complicates this conclusion. All attempts to clone β-galactosidase-harpin fusion proteins have been unsuccessful, as were attempts to clone and express *hrpN* downstream of the *lacZ* promoter in several commonly used vectors such as pBluescript. Expression of such constructs is evidently deleterious to bacterial strains.

Wei, et al., “Harpin, Elicitor of the Hypersensitive Response Produced By the Plant Pathogen *Erwinia amylovora,*” *Science,* 257:85-88 (1992), which is hereby incorporated by reference, reported previously that BLAST searches indicated harpin$_{Ea}$ possessed slight similarity to several other glycine-rich proteins, including
keratins and glycine-rich cell wall proteins. However, this is thought to be due to the high glycine content of harpin\textsubscript{Ea}, and as such does not suggest a role for harpin\textsubscript{Ea}. Examination of N-terminal sequences from several HR-eliciting proteins produced by phytopathogenic bacteria (Figure 8) yielded some potential similarities. However, the region in question is quite short. The region of putative primary sequence similarity is limited to the first 26 residues at the N-terminus, and its role remains unclear.

Surprisingly, \textit{E. carotovora} harpin\textsubscript{Eec} appears more similar to the harpins from \textit{E. amylovora} and \textit{E. stewartii} than to that from \textit{E. chrysanthemi}, to which it is more closely related with respect to its taxonomic position as well as its mechanism of pathogenicity (i.e. soft-rots). In addition, although primary sequence similarity is strongest only in the C-terminal third of each protein, the \textit{Erwinia} harpins possess broadly similar hydrophobicity profiles along their entire lengths (Figure 9). Based on its hydrophobicity profile, Alfano, et al., “Analysis of the Role of the \textit{Pseudomonas syringae} HrZ harpin in Elicitation of the Hypersensitive Response in Tobacco Using Functionally Nonpolar hrZ Deletion Mutations, Truncated HrZ Fragments, and \textit{hrm4} Mutations,” \textit{Mol. Microbiol.} 19:715-728 (1996), which is hereby incorporated by reference, speculated that harpin\textsubscript{Ps} may have an amphiphilic nature. However, the \textit{Erwinia} harpins’ profiles do not match that of harpin\textsubscript{Ps}.

Proteinaceous HR-elicitors have also now been described from *Phytophthora secalis* (Rohe, et al., “The Race-Specific Elicitor, NIP1, From the Barley Pathogen, *Rhynchosporium secalis*, Determines Avirulence on Host Plants of the *Rrs1* Resistance Genotype,” EMBO Journal 14:4168-4177 (1995) which is hereby incorporated by reference, while *P. infestans* (Pieterse, et al., “Structure and Genomic Organization of the *ipiB* and *ipiO* Gene Clusters of *Phytophthora infestans*,” Gene, 138:67-77 (1994), which is hereby incorporated by reference) produces a glycine-rich pathogenicity-associated family of proteins of unknown function. Because the primary amino acid sequence of each elicitor protein or peptide fragment shows no obvious similarity to that of the others, it is unclear whether they interact with the same target on or in the plant cell, plasma membrane, or cell wall. In that regard, it might be of interest to test whether any one of these molecules inhibits the action of other(s). The increasing availability of peptides such as Pep13, Avr9, P68, and harpin*<sub>EA</sub>* C305 with plant-defense response-eliciting activity (HR and otherwise) should enable precise probing of their targets on or in plant cells, as well as determination of whether their mechanisms of activity are similar, distinct, or overlapping.

**Example 14 - Bacterial strains and plasmids**

*Escherichia coli* stains used in the following examples include DH5α and BL21(DE3) purchased from Gibco BRL and Stratagene, respectively. The pET28(b) vector was purchased from Novagen. Eco DH5α/2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at the Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)<sub>6</sub> vector pET28(b) which contained a Kan<sup>r</sup> gene for selection of transformants.

**Example 15 - DNA Manipulation**

Restriction enzymes were obtained from Boehringer Mannheim or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR
Supermix™ were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR Purification Kit were purchased from Qiagen. The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (molecular cloning) or protocols provided by the manufacturer.

**Example 16 - Fragmentation of hrpN gene**

A series of N-terminal and C-terminal truncated hrpN genes and internal fragments were generated via PCR (Fig. 10). The full length hrpN gene was used as the DNA template and 3’ and 5’ primers were designed for each truncated clone (Fig. 11). The 3’ primers contained the NdeI enzyme cutting site which contained the start codon ATG (Methionine) and the 5’ primers contained the stop codon TAA and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp™ 9600 or 9700. 45 µl of Supermix™ were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and diH2O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex). Amplified DNA was purified with the QIAquick PCR purification kit, digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9 ml SOC solution or 0.45 ml LB media
were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit. The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pet28(b) vector was generated as a positive control, and a clone with only the pET28(b) vector was generated as a negative control.

**Example 17 - Expression of harpin truncated proteins**

*Escherichia coli* BL21(DE3) strains containing the hrpN clones were grown in Luria broth medium (g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at 37°C to an OD₆₂₀ of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD₆₂₀ of ca. 0.3 or 0.6-0.8. One milli molar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH₂PO₄, and 0.72 K₂HPO₄), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 2).
Table 2: Expression of harpin truncated proteins

<table>
<thead>
<tr>
<th>Fragment</th>
<th>amino acids (SEQ. ID. No. 23)</th>
<th>Growth medium</th>
<th>Induction O.D.</th>
<th>Expression temp.</th>
<th>Harvest time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ control)</td>
<td>1-403</td>
<td>LB</td>
<td>ca. 0.3 or 0.6-0.8</td>
<td>19°C or 25°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>2 (+ control)</td>
<td>-</td>
<td>LB and TB</td>
<td>ca. 0.3 or 0.6-0.8</td>
<td>19°C and 37°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>3</td>
<td>105-403</td>
<td>LB</td>
<td>0.6-0.8</td>
<td>19°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>4</td>
<td>169-403</td>
<td>TB</td>
<td>ca. 0.3</td>
<td>19°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>5</td>
<td>210-403</td>
<td>LB or M9ZB</td>
<td>0.6-0.8</td>
<td>19°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>6</td>
<td>257-403</td>
<td>LB or M9ZB</td>
<td>0.6-0.8</td>
<td>19°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>7</td>
<td>343-403</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>19°C</td>
<td>5 hr</td>
</tr>
<tr>
<td>8</td>
<td>1-75</td>
<td>TB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>9</td>
<td>1-104</td>
<td>TB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>10</td>
<td>1-168</td>
<td>TB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>11</td>
<td>1-266</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>4 hr</td>
</tr>
<tr>
<td>12</td>
<td>1-342</td>
<td>LB</td>
<td>0.6-0.8</td>
<td>19°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>13</td>
<td>76-209</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>5 hr</td>
</tr>
<tr>
<td>14</td>
<td>76-168</td>
<td>TB or LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3 hr or 16-18 hr</td>
</tr>
<tr>
<td>15</td>
<td>105-209</td>
<td>M9ZB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3 hr</td>
</tr>
<tr>
<td>16</td>
<td>169-209</td>
<td>no expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>105-168</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3-5 hr</td>
</tr>
<tr>
<td>18</td>
<td>99-209</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3 hr</td>
</tr>
<tr>
<td>19</td>
<td>137-204</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3 hr</td>
</tr>
<tr>
<td>20</td>
<td>137-180</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>16-18 hr</td>
</tr>
<tr>
<td>21</td>
<td>105-180</td>
<td>LB</td>
<td>ca. 0.3</td>
<td>37°C</td>
<td>3 hr</td>
</tr>
<tr>
<td>22</td>
<td>150-209</td>
<td>no expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>150-180</td>
<td>no expression</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

General expression method: Escherichia coli BL21(DE3) strains containing the hrpN subclones were grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5g/L NaCl, and 1 mM NaOH) containing 30 μg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at 37°C to an OD620 of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the growth medium and grown at 37°C to a specific induction OD620. One milli molar IPTG was then added and the cultures grown at an optimal temperature for protein expression, and harvested at a particular time for recovery of the highest level of protein.

**Example 18 - Small scale purification of harpin truncated proteins (verification of expression)**

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na2HPO4, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant
saved. A 50 μl aliquot of a 50% slurry of an equilibrated (His)_6-binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na_2HPO_4, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 μl of urea elution buffer (8 M urea, 0.1 M Na_2HPO_4, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3). The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

**Example 19 - Induction of HR in tobacco**

A 1.5 ml aliquot from the 50 ml cultures grown for small scale purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

**Example 20 - Large scale native purification of harpin truncated proteins for comprehensive biological activity assays**

Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, and incubated at 30°C for 15 minutes, sonicated for two minutes, then centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4
ml aliquot of a 50% slurry of an equilibrated (His)$_6$-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 21 - Large scale urea purification of harpin truncated proteins for comprehensive biological activity assay

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.
Example 22 - Expression of harpin truncated proteins

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 3).

Table 3

Expression and HR activity of harpin truncated proteins (small scale screening)

<table>
<thead>
<tr>
<th>Fragment #</th>
<th>Amino Acids (SEQ. ID. No. 23)</th>
<th>Expression</th>
<th>HR activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ control)</td>
<td>1-403</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (- control)</td>
<td>-</td>
<td>background protein only</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>105-403</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>169-403</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>210-403</td>
<td>+</td>
<td>-</td>
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<td>6</td>
<td>267-403</td>
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<td>7</td>
<td>343-403</td>
<td>+/-</td>
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<td>+</td>
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<tr>
<td>13</td>
<td>76-209</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>76-168</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
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</tr>
<tr>
<td>16</td>
<td>169-209</td>
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<tr>
<td>17</td>
<td>105-168</td>
<td>+</td>
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<tr>
<td>18</td>
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<tr>
<td>22</td>
<td>150-209</td>
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</tr>
<tr>
<td>23</td>
<td>150-180</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All of the cloned fragment proteins were expressed to a certain degree except for three small fragments (amino acids 169-209, 150-209, and 150-180). The fragments were expressed at varying levels. Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and
negative control proteins, consisting of the full length harpin protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 4).

### Table 4

Expression level and HR titer of harpin truncated proteins (large scale purification)

<table>
<thead>
<tr>
<th>Fragment #</th>
<th>Amino acids (SEQ. ID. No. 23)</th>
<th>Expression</th>
<th>HR titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ control)</td>
<td>1-403</td>
<td>3.7 mg/ml</td>
<td>5-7 µg/ml</td>
</tr>
<tr>
<td>2 (- control)</td>
<td>-</td>
<td>-</td>
<td>1:2 dilution</td>
</tr>
<tr>
<td>4</td>
<td>169-403</td>
<td>2 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>210-403</td>
<td>5 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>267-403</td>
<td>4 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>345-402</td>
<td>200 µg/ml</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1-75</td>
<td>50 µg/ml</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1-104</td>
<td>50 µg/ml</td>
<td>3 µg/ml (1:16 dilution)</td>
</tr>
<tr>
<td>10</td>
<td>1-168</td>
<td>1 mg/ml</td>
<td>1 µg/ml</td>
</tr>
<tr>
<td>13</td>
<td>76-209</td>
<td>2.5 mg/ml</td>
<td>5 µg/ml</td>
</tr>
<tr>
<td>14</td>
<td>76-168</td>
<td>2 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>105-209</td>
<td>5 mg/ml</td>
<td>5-10 µg/ml</td>
</tr>
<tr>
<td>17</td>
<td>105-168</td>
<td>250 µg/ml</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>137-204</td>
<td>3.6 mg/ml</td>
<td>3.5 µg/ml</td>
</tr>
<tr>
<td>20</td>
<td>137-180</td>
<td>250 µg/ml</td>
<td>16 µg/ml</td>
</tr>
</tbody>
</table>

Not all of the proteins were expressed in large scale due to time constraints. The truncated proteins deemed to be the most important in characterizing harpin were chosen. The positive control (full length harpin) was expressed in a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also, however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The negative control contained several background proteins as expected, but no obviously induced dominant protein.
Example 23 - Induction of HR in tobacco

The full length positive control protein elicited HR down to only 5-7 μg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

One definitive domain encompassed a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

The internal domain proteins elicited an HR response between 5 and 10 μg/ml of protein like the positive control, and the N-terminus domain proteins elicited an HR response between 1 and 3 μg/ml, lower than the positive control.

Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.
The harpin C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, the protein between a.a. 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit the HR response. It was not expected that there would be an HR response from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR response. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR. It is also possible, although unlikely, that these small proteins would have undergone some form of post-translational modification within the E.coli cell that they did not contain when synthesized and, therefore, were not able to elicit an HR response.

Example 24 – Biological Activity of HR Inducing Fragments

The two N-terminal harpin fragments spanning nucleotides 1-104 and nucleotides 1-168 of the nucleic acid of SEQ. ID. No. 24 were effective at inducing resistance of tobacco against TMV, in a similar manner as the full length harpin protein. The internal fragments spanning nucleotides 76-209 and nucleotides 105-209 of the nucleic acid of SEQ. ID. No. 24 were also effective at inducing TMV resistance. In addition, these same four fragments conferred plant growth enhancement ("PGE") in tomato increasing the height of the plants from 4-19% taller than the buffer control plants. The full length harpin protein induced growth
enhancement of 6% greater than the buffer. The negative control did not induce TMV resistance or growth enhancement.

Table 5

<table>
<thead>
<tr>
<th>Fragment #</th>
<th>Amino acids (SEQ. ID. No. 23)</th>
<th>HR activity</th>
<th>TMV resistance</th>
<th>PGE ht &gt; buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ control)</td>
<td>1-403</td>
<td>+</td>
<td>+</td>
<td>6%</td>
</tr>
<tr>
<td>2 (- control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-2%</td>
</tr>
<tr>
<td>9</td>
<td>1-104</td>
<td>+</td>
<td>+</td>
<td>4-8%</td>
</tr>
<tr>
<td>10</td>
<td>1-168</td>
<td>+</td>
<td>+</td>
<td>5-13%</td>
</tr>
<tr>
<td>13</td>
<td>76-209</td>
<td>+</td>
<td>+</td>
<td>4-18%</td>
</tr>
<tr>
<td>15</td>
<td>105-209</td>
<td>+</td>
<td>+</td>
<td>6-19%</td>
</tr>
</tbody>
</table>

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cornell Research Foundation, Inc. and EDEN Bioscience Corporation

(ii) TITLE OF INVENTION: HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS ELICITING A HYPERSENSITIVE RESPONSE AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
(B) STREET: Clinton Square, P.O. Box 1051
(C) CITY: Rochester
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 14603

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/048,109
(B) FILING DATE: 30-MAY-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Goldman, Michael L.
(B) REGISTRATION NUMBER: 30,727
(C) REFERENCE/DOCKET NUMBER: 19603/1302

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (716) 263-1304
(B) TELEFAX: (716) 263-1600

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAATTCCAT ATGAGTCTGA ATACAAGTGG G

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGGAATTCCAT ATGGGCGGTG GCCAGGCGG T

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCATATGTC GAACGCCTG AAGGATATG

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGAATTCCAT ATGGGCGGTG GCCAGGCTAA C

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 29 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGCATATGCT GAACAGCTG GGCTCGAAA

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 29 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCATATGTC AACGTCAGAA AACGACGAT

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 27 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCATATGTC CACCTCAGAC TCCAGCG

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 34 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GGGAATTCA ATGCAAAGCC TGTGGGTGTA TGGG

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GGGAATTCA ATGGGAATGA GTCTGAGCAA G

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GGGAATTCA ATGAAAGCGG GCATCGAGGC G

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GGGAATTCA ATGACACCAG CCAGTATGGA GCAG
(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:12:
GCAAGCCTAA CAGCCCAACCCA GCGCCCATCA T

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:13:
GCAAGCCTTAATCGTTTACGC CGTTCGACA G

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:14:
GCAAGCCTTAATCTCGCTG AACATCTTCAG CAG

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
GCAAGCTTAA GGTGCCATCT TGCCCATCAC

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
GCAAGCTTAA ATCAGTGACT CCTTTTTAT AGGC

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
GCAAGCTTAA CAGGCCCCAC AGCGCATCAG T

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
GCAAGCTTAA ACCGATAACG GTACCACGG C
(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCAAGCTTTAA TCCGTCGTCATCTGGCTTGCTCAG

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCAAGCTTTAA GCCGCGCCTA GCTTG

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 338 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1 5 10 15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
35 40 45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
50
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65
70
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
85
90
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100
105
Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115
120
125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130
135
140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ser Ile Leu Gly
145
150
155
160
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165
170
175
 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Gly Ala Phe Asn Gln Leu
180
185
190
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Leu Ser Ala
195
200
205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210
215
220
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225
230
235
240
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245
250
255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260
265
270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275
280
285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290
295
300
Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305
310
315
320
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
325
330
335
Asn Ala
(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGATTTTACC CGGGTGAAAC TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG 60
GGTTTTATGG CGGCCATGAA CGGGCATCAG GGCGCGCGCT GGTGGCGGCA ATCCGGCGTC 120
GATCTGTGAT TTCACTTTGG GACACCGCGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG 180
CAGCAATATCC GGCGCATGTG GCGCAAGCTC CTAGCTGTCG GTATCAGCAG GCGGCGAGAG 240
TGCGAGCAGTCT GGGCATCTGTC CTGGAACGGC AGCGATGTAT TGATCCTCTG TGGGCCGCTG 300
CCGTCGGATC CGCCAGGTTA TCCGAGGTTA ATCGAACGTT TGTTGAACCT GGGCGGAATG 360
ACGTTGCCGT CGCTATCCAT AGCACCGGAC GCGGTCGCGC AGACACGAGA CCGACGGGCC 420
CGATCATCAA GATATAGGCC GCTTTTTTTA TTGCAAAACG GTAACCGGTGA GGAACCGTTT 480
CACCGTCGGC GTCACTCAGT AAACAGTATC CATCAGATCG CTCACATCGG GATCGCGCTG 540
GGCATCCCGT GGAGATACCT TTTGGAACAC CTGACATGAA TGAGAAACG AAATTAAGCA 600
AATTAGCAGAT CAAAGCGCACA TGCGGGCCTGA TTTGGCGGTC TCCGCGCTGG GGGCTGGGTC 660
TCAGGGACTG AAAGGACTGA ATTCGGCGCG TTCTCGCGTG GGGTGACCGAC TGGATGAACGT 720
GAGCAGCACCC ATCGATACGT TGACCTCGGC GCTGACTTCG ATGATGGTG GCGCGCGCCT 780
GGCGAGGGGG CTTGGCGGCGC GTCGAAGGGG GGTGAGGGATG AGCAACTAAC TGGCCCGAGTC 840
TTTCCGGAAT GCGCCGCGAG ATGGGCACCA CTCGCTATCC GTACCAGAAT CGGCGCGGCA 900
TGCTGTGTCAT AAAATGTTTG ATAAAGCGCT GGAGGCTCTG CTGCTGCTAG ACACCGTGAC 960
CAAGCTGACT ACCAGAGAC ACCAACTGTC TAATTCCAAAT CTGAAACGCCA GCGAGATGAC 1020
CCAGGATATT ATGAATGCCT TGCGCAGCGG TGGAACAACG GCACGTGCTG CCATTCTCGG 1080
CAAGCTTCTG GCGCACTGA TGATGCGGCT CTCTCGCCCT TCTCGCCCCGG CAGCGCGCTT 1140
GCGGGCGCTTG AGCGCGCGAG GTGCATTCAG CAGTTGGGAT AATGCCATCG GCATGGGGCGT 1200
GGGCGAGAAT GCTGCGCGTA GTCCGCTTGA TAGCCTGACG ACACCGTACG AGCGTAACAA 1260
(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
1    5    10    15

Ile Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
20   25    30

Asn Ala Gly Leu Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
35   40    45
Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385 390 395 400

Gly Ala Ala

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAGCTTCGGC ATGGCAGGTT TGACCCTTTG TGCAGCTCgg GTCGGCGAGG TACGTTTGA TTATTCATAA 60
GAGGAATACG TTATGACTTT GATACACAGT GGGCTGGGAG GTCAACGAT GCAAAATTTC 120
ATCGGCGGAG CGGCGGAAA TAAACCGTGT CCGGCTACCA GTCGCCAGAA TGCTGGGTTG 180
GCTGGCAGAT CTGCACTGGG GCTGCCCAGGC GGTAATAAAA ATGATAACCT GAATCAGCTG 240
GCTGCGTTAC TCACCGGCGAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGCCGCTGATG 300
GCCGGGGTGGT AGGGGCAGGG TTGGGCGGTGG GCTCACCGTGG CCGGCGGAA 360
GGACTGTGCA AGCCGCTGAA CGATATGTTA GCCGGCTCGC TGAACAAGCT GGGCTCGAAA 420
GGCGCAACA ATACCACTTC AAACAACAAAT TCCCCGCTGG ACCAGGCCGCT GGGTATTAAC 480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCAACCTCAGA TCCAGCAGAC 540
CCGATGCGAC AGCTGCTGAA GATGTCACG GAGATAATGC AAAGGCTTTT TGCTGATGGG 600
CAAGATGGCAG CCGGGCGAG TTCCCTCGTG GGCAACAGAC CAGCCCGAAG CGACGAGAAC 660
GCCTATAGAA AAGGATCAG TGATGCGGCTG TGGGCGCTGA TGGTAAATGG TGGAGGCGAG 720
CTCCTTTGCA ACGGGGACT GGGAGCTGT CATGGCGCTTA ATGCTGGCAC GGGCTTTAC 780
(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1      5      10     15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20     25     30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35     40     45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50     55     60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65     70     75     80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85     90     95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Gln Asp Leu Met
100    105   110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115    120   125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
   130       135       140
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Pro Ala Gln Phe Pro  
   145       150       155       160
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
   165       170
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
   180       185       190
Gly Gln Gln Leu Gln Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
   195       200       205
Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
   210       215       220
Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
   225       230       235       240
Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
   245       250       255
Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val  
   260       265       270
Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
   275       280       285
Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala  
   290       295       300
Thr Leu Lys Asp Ala Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
   305       310       315       320
Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
   325       330       335
Asn Gln Ala Ala Ala  
   340

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1026 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

ATGCAGAGTC TCAGTCTTTAA CAGCAGCTCG CTGCAAACC CGGCAATGGC CTTTGCTCTG 60
GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCAAGCA AGGCCAAGTTA GGAAGTTGTC 120
GTGAAGCTGG CGCGAGAATC GATGGCGAAAT GTGCAACTCG ACGACAGCTC GCCATTGGGA 180
AAACTGTTGG CCAAGTCCAT GGCCCGAGAT GGCAAGGCGG GCAGCGCTAT TGGAGATGTC 240
ATCCAGCGGC TGACAAGCTG CATCATGAAA AAGCTCAGTG ACAACTTCCG CGCGTCTCGG 300
GACAGCGCCT CGTACCAAGG ACAAGCGGAC CTGATGACTC AGTGCTCAAA TGCCCTGGCC 360
AAGTCGATGC TCGATGATCT TCGAAGTCAG CAGAGACCCG GGAAGACCTT CTCCGAAAGC 420
GATATGCGGA TGCTGACACGA GATCGGCCAG TCTAGGATGC ACAATCCGGC AGAATTTCCC 480
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GAAACGCGTG CTGTTCCGCTC GCACATGCAG ATGATTGGCC AGCACTGCGG TAAATCGCAG 600
AGTGCAGCTG GCAGTCTGCG AGGGAGGCTG GGAGGTCGAG GCACCTCGAG CAGTTTTTCC 660
AACAACCTGT CGTCTAGGG TGATCAGCTG ATCAGACGCA ATACCGCGTC CGTGGACAGC 720
GGCAATACCC GCTGGTGAGCC GGGGCAATGG ATCAGCGCCG TTATCGACCG TGCCCTGCAA 780
TCGTTATTGG CGGTGGTGGT ACTGCGCCAA CCCATTAACA CCCCAGAGAC CGSTACGTCG 840
GCGAATGGGC GACATGCGGC TCAGGCTTCT GTACGTTGCA TGGCCGCTTT TGGCTCAAG 900
GGCGCTGGAG CCAGCGCTCA AAGAGCAGCG CCGAGCGTGA GTGACGCCGCT 960
GCGCAAATCG CCACCTGCTG GTGCTAGTAC CGTGCTCAAG GCACCCCGCAA TGAGGCTGCA 1020
GCCTGA 1026

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 344 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln

1      5      10     15
Asn Leu Asn Leu Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
  20  25  30
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
  35  40  45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
  50  55  60
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
  65  70  75  80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
  85  90  95
Ala Asn Lys Thr Gly Asn Val Asp Ala Asn Asn Gln Asp Pro Met
  100 105 110
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
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Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
  130 135 140
Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gly Gly Gly Leu Ala
  145 150 155 160
Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
  165 170 175
  180 185 190
Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
  195 200 205
Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
  210 215 220
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Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln 325 330 335
Gln Ser Thr Ser Thr Gln Pro Met 340

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1035 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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AACACCAACA CCAACAGGCA GCAATCGGCC CAGTCCGGTC AAGACCTGAT CAAGCAGGTC 120
GGAAAGGACA TCCCTCAACT CATCGCAGGC CTGCATCGGA AGGCCGCAAC GTGCGCGGCC 180
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GAAGGCCTGC AGGGAGTCCA CGAGATCCTC GCCCAGCTCG GCAGCGCGGG TGCTGCGGCC 540
GGCCCGGCCG GTGCAGGGTG GCCCGGGCTC GGTGCAGGGG ATGGCGGGCTC CCGTGGCGGT 600
GGGCAGCGGC GTGCAGGGGC GCGCGCGGCC GCAATGCGGC TGAACGCGCA CCAGCGCAAC 660
GGCCCGCAGA AGCACGCGGA TGTCACAAGGT GCGAAGGGCG CGATGACGCC CAGCGAGAC 720
CAGGGCGGCC TCACCGCGGT GCTGCAAAGG CTGATGCAAG TCTGGAAGGC GCTGCGCGAG 780
ATGCATGACG AAGCCCGCCT CGCGCGCGCC AACCAGCGGC AGGCGCGGCTC GAAGGTGCCC 840
GGCAACGCCT CGCCCGCGTC CGCCCGCGAC CGCCCGCGGC ACCAGCGCGC TTCGGCGCGAT 900
GATCAATCGT CGGCCAGAA CAATCTGCAA TCCCATGACTA TGGATGCGGT GAAGGAGGTC 960
GTCCAGATCC TGCAGCAGAT GCTGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020
ACGCAGCCGA TGTA 1035

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:29:
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1 5 10 15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:30:
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20
WHAT IS CLAIMED:

1. An isolated fragment of an *Erwinia* hypersensitive response elicitor protein or polypeptide, wherein said fragment elicits a hypersensitive response in plants.

2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, or *Erwinia stewartii*.

3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ-ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning amino acids 105 and 403 of SEQ. ID. No. 23.

6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372.

7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.
8. An isolated DNA molecule encoding a fragment according to claim 1.

9. An isolated DNA molecule according to claim 8, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, or *Erwinia stewartii*.

10. An isolated DNA molecule according to claim 9, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

11. An isolated DNA molecule according to claim 10, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

12. An isolated DNA molecule according to claim 10, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning amino acids 105 and 403 of SEQ. ID. No. 23.

13. An isolated DNA molecule according to claim 10, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372.

14. An isolated DNA molecule according to claim 10, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.
15. An expression system transformed with a DNA molecule according to claim 8.

16. An expression system according to claim 15, wherein said DNA molecule is in proper sense orientation and correct reading frame.

17. A host cell transformed with a DNA molecule according to claim 8.

18. A host cell according to claim 17, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

19. A host cell according to claim 17, wherein the DNA molecule is transformed with an expression system.

20. A transgenic plant transformed with the DNA molecule of claim 8.

21. A transgenic plant according to claim 20, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

22. A transgenic plant according to claim 20, wherein the plant is selected from the group consisting of Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

23. A transgenic plant seed transformed with the DNA molecule of claim 8.
24. A transgenic plant seed according to claim 23, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

25. A transgenic plant seed according to claim 23, wherein the plant seed is selected from the group consisting of Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

26. A method of imparting disease resistance to plants comprising:
applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions impart disease resistance.

27. A method according to claim 26, wherein plants are treated during said applying.

28. A method according to claim 26, wherein plant seeds are treated during said applying, said method further comprising:
planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

29. A method of enhancing plant growth comprising:
applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.
30. A method according to claim 29, wherein plants are treated during said applying.

31. A method according to claim 29, wherein plant seeds are treated during said applying, said method further comprising:
   planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and
   propagating plants from the seeds planted in the soil.

32. A method of insect control for plants comprising:
   applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to control insects.

33. A method according to claim 32, wherein plants are treated during said applying.

34. A method according to claim 32, wherein plant seeds are treated during said applying, said method further comprising:
   planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and
   propagating plants from the seeds planted in the soil.

35. A method of imparting disease resistance to plants comprising:
   providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, and
   growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to impart disease resistance.

36. A method according to claim 35, wherein a transgenic plant is provided.
37. A method according to claim 35, wherein a transgenic plant seed is provided.

38. A method of enhancing plant growth comprising:

providing a transgenic plant or a plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, and

growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to enhance plant growth.

39. A method according to claim 38, wherein a transgenic plant is provided.

40. A method according to claim 38, wherein a transgenic plant seed is provided.

41. A method of insect control for plants comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment elicits a hypersensitive response, and
growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to control insects.

42. A method according to claim 41, wherein a transgenic plant is provided.

43. A method according to claim 41, wherein a transgenic plant seed is provided.
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Fragments separately sufficient for HR-eliciting activity

**FIGURE 1**
Figure 5A

Figure 5B

Activity: + + + +

FIGURE 5A-B
SMEE (metalloproteinase) cleavage sites
Endopeptidase Glu-C cleavage sites (Glu)
Trypsin cleavage sites
Active fragments

↑ Cleavage at these sites abolishes HR eliciting activity of harpin C305 and of peptides 764-768 and 917-919.

FIGURE 7
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*aEch= E. chrysanthemi; Ecc= E. carotovora subsp. carotovora; Eam = E. amylovora; Est= E. stewartii.*
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**FIGURE 10**
N1; 5'-GGGAATTCAATATGAGTCTGAATAACAGTG-3'
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N110; 5'-GGCTATATGCTGAACAGCTCAGTGATATG-3'
N137; 5'-GGCTATATGCTGAACAGCTCAGTGATATG-3'
N150; 5'-GGCTATATGCTGAACAGCTCAGTGATATG-3'
N169; 5'-GGGAATTCTATATGGAAAGCTTTTGGATAGG-3'
N210; 5'-GGGAATTCTATATGGAAAGCTTTTGGATAGG-3'
N267; 5'-GGGAATTCTATATGGAAAGCTTTTGGATAGG-3'
N343; 5'-GGGAATTCTATATGGAAAGCTTTTGGATAGG-3'
C75; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C104; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C168; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C180; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C204; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C209; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C266; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C342; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'
C403; 5'-GCAAGCTTTAACAGGCACCCAGCCCTCATCA-3'

FIGURE 11