



(72) CRANE, VIRGINIA C., US

(71) PIONEER HI-BRED INTERNATIONAL, INC., US

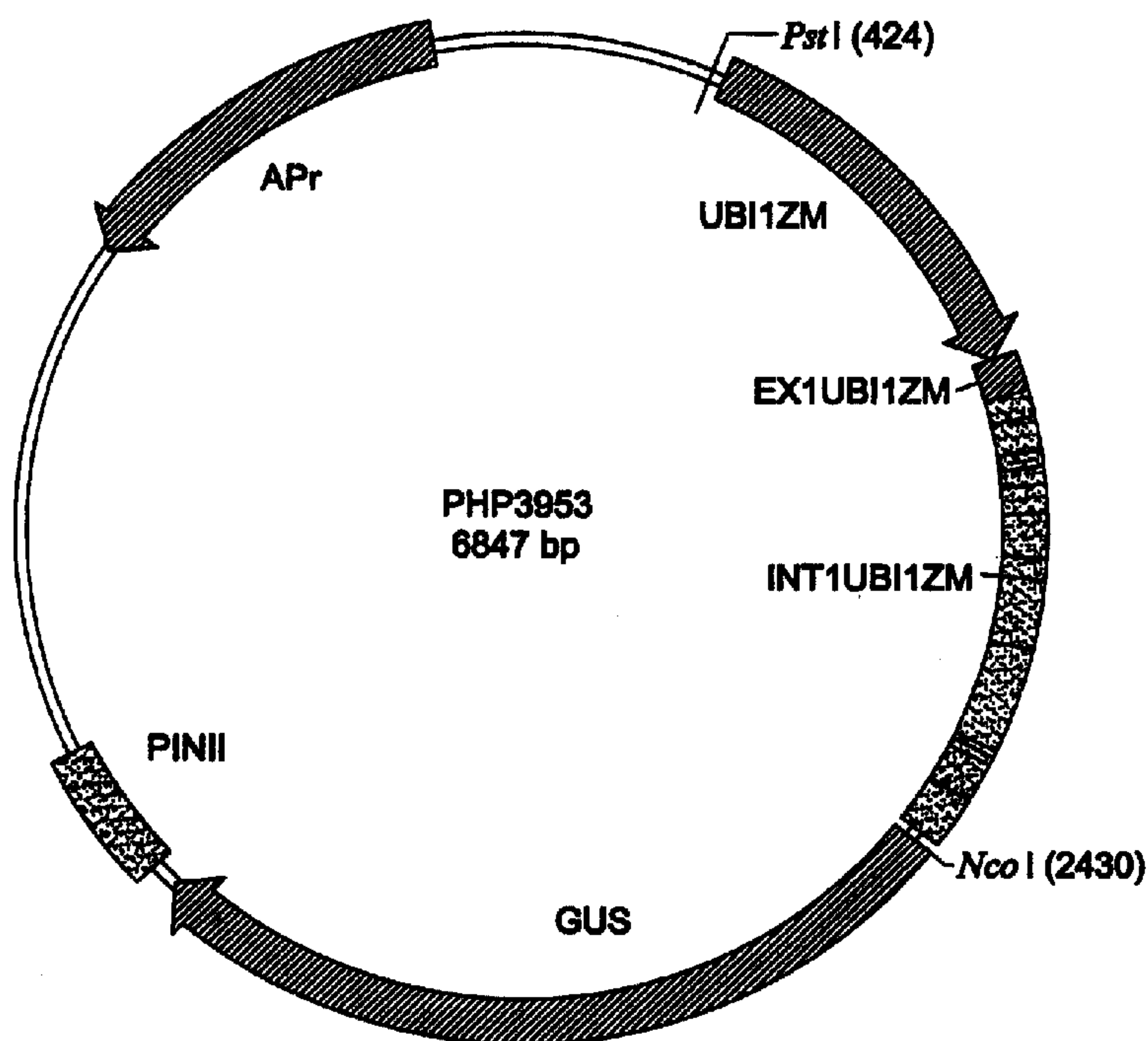
(51) Int.Cl.<sup>6</sup> C12N 15/29, C12N 15/82, C07K 14/415, A01H 5/00, C12N 5/10,  
A01H 5/10

(30) 1998/02/26 (60/076,100) US

(30) 1998/03/27 (60/079,648) US

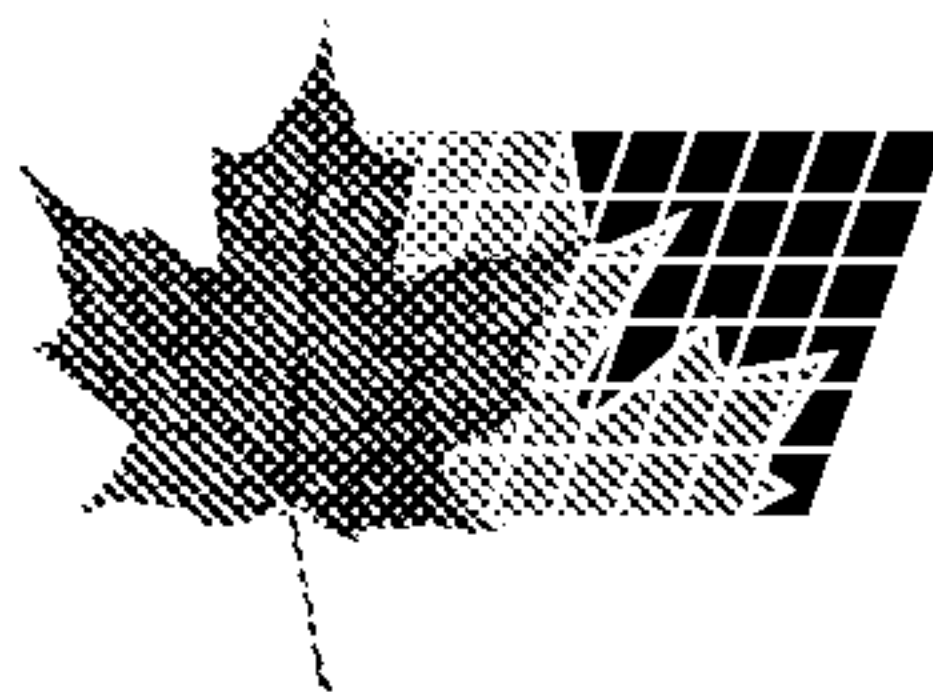
(54) **FAMILLE DE GENES PR-1 ET DE PROMOTEURS**

(54) **FAMILY OF MAIZE PR-1 GENES AND PROMOTERS**



(57) La présente invention concerne des compositions et des méthodes de régulation de l'expression de séquences nucléotidiques hétérologues dans une plante. Les compositions sont de nouvelles séquences de nucléotides pour des promoteurs inductibles et constitutifs isolés d'une famille de gènes du maïs codant des protéines liés

(57) The present invention provides compositions and methods for regulating expression of heterologous nucleotide sequences in a plant. Compositions are novel nucleotide sequences for inducible and constitutive promoters isolated from a family of maize genes encoding pathogenesis-related (PR-1) proteins,



(21) (A1) **2,315,549**  
(86) 1999/02/11  
(87) 1999/09/02

à la pathogénèse (PR-1), des séquences de nucléotide pour ces gènes PR-1 du maïs, ainsi que des séquences d'acides aminés pour les protéines PR-1 ainsi codées. L'invention a également trait à des méthodes de régulation de l'expression d'une séquence nucléotidique hétérologue dans une plante de manière inductible et constitutive, plus particulièrement de régulation de l'expression d'un gène PR-1 pour augmenter la résistance aux maladies dans une plante. Les méthodes consistent à transformer une cellule végétale afin qu'elle comprenne une séquence nucléotidique hétérologue ou un gène PR-1 lié fonctionnellement à un des promoteurs inductibles ou constitutifs de la présente invention, et à régénérer une plante transformée de manière stable à partir de la cellule végétale transformée. L'invention concerne également les plantes et les semences transformées présentant une résistance accrue aux maladies.

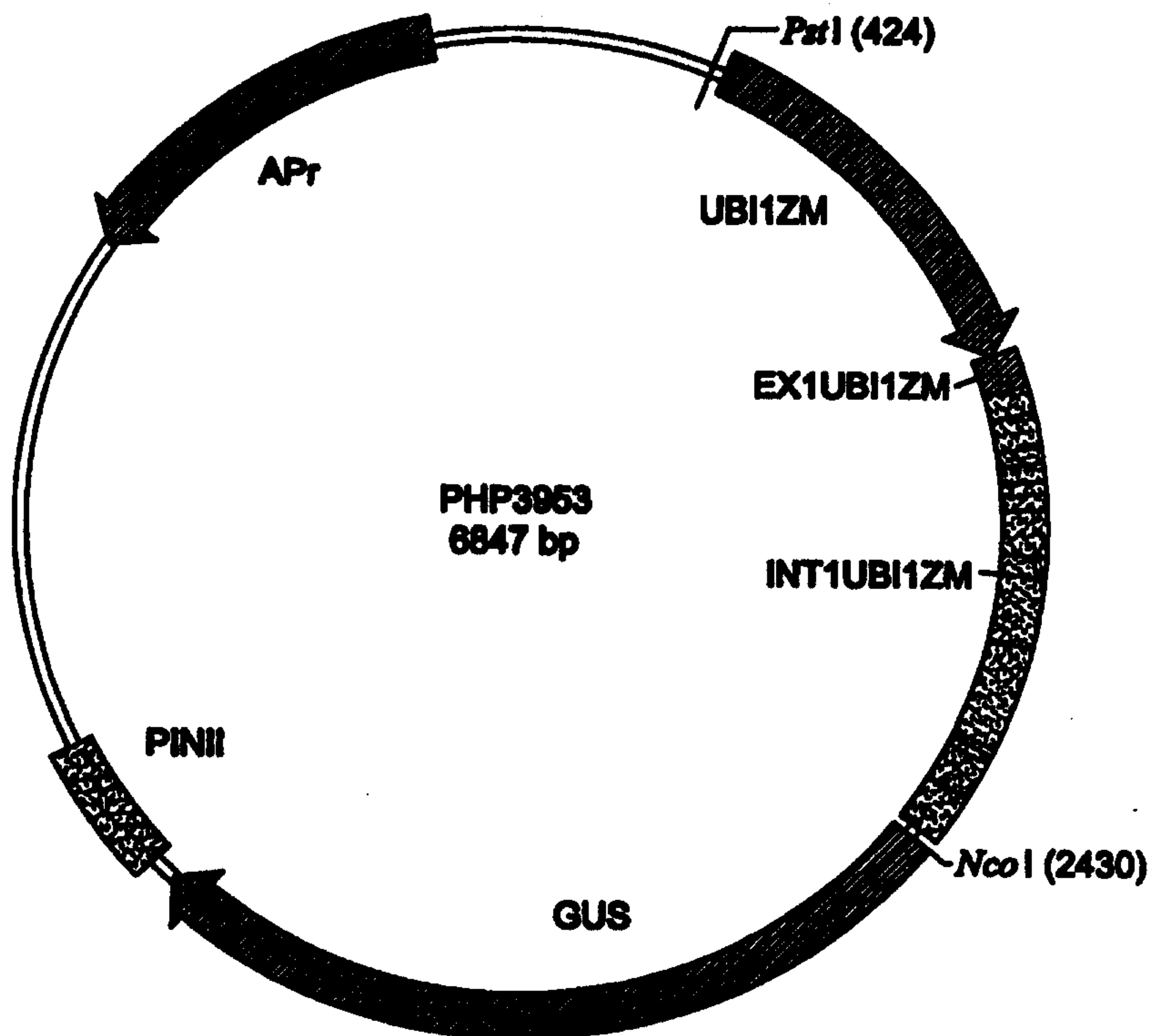
nucleotide sequences for these maize PR-1 genes, and amino acid sequences for the PR-1 proteins encoded thereby. Methods for regulating expression of a heterologous nucleotide sequence in a plant in an inducible or constitutive manner are provided, more particularly regulating expression of a PR-1 gene to enhance disease resistance in a plant. The methods comprise transforming a plant cell to comprise a heterologous nucleotide sequence or PR-1 gene operably linked to one of the inducible or constitutive promoters of the present invention and regenerating a stably transformed plant from the transformed plant cell. Transformed plants and seeds having enhanced disease resistance are also provided.



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12N 15/29, 15/82, 5/10, C07K 14/415,</b><br><b>A01H 5/00, 5/10</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/43819</b><br><b>(43) International Publication Date:</b> 2 September 1999 (02.09.99)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>(21) International Application Number:</b> PCT/US99/03011<br><b>(22) International Filing Date:</b> 11 February 1999 (11.02.99)<br><b>(30) Priority Data:</b><br>60/076,100           26 February 1998 (26.02.98)   US<br>60/079,648           27 March 1998 (27.03.98)    US<br><b>(71) Applicant (for all designated States except US):</b> PIONEER<br>HI-BRED INTERNATIONAL, INC. [US/US]; 800 Capital<br>Square, 400 Locust Street, Des Moines, IA 50319 (US).<br><b>(72) Inventor; and</b><br><b>(75) Inventor/Applicant (for US only):</b> CRANE, Virginia, C.<br>[US/US]; 5052 Ingersoll Avenue, Des Moines, IA 50312<br>(US).<br><b>(74) Agents:</b> SPRUILL, W., Murray et al.; Alston & Bird LLP, P.O.<br>Drawer 34009, Charlotte, NC 28234-4009 (US).                                                                                                                                                                                                                                                                                                                                                                                |           | <b>(81) Designated States:</b> AL, AM, AT, AT (Utility model), AU, AZ,<br>BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility<br>model), DE, DE (Utility model), DK, DK (Utility model),<br>EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD,<br>GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP,<br>KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,<br>MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI,<br>SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US,<br>UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW,<br>SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ,<br>MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE,<br>DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE),<br>OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML,<br>MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the</i><br><i>claims and to be republished in the event of the receipt of</i><br><i>amendments.</i> |
| <b>(54) Title:</b> FAMILY OF MAIZE PR-1 GENES AND PROMOTERS<br><br><b>(57) Abstract</b><br><br><p>The present invention provides compositions and methods for regulating expression of heterologous nucleotide sequences in a plant. Compositions are novel nucleotide sequences for inducible and constitutive promoters isolated from a family of maize genes encoding pathogenesis-related (PR-1) proteins, nucleotide sequences for these maize PR-1 genes, and amino acid sequences for the PR-1 proteins encoded thereby. Methods for regulating expression of a heterologous nucleotide sequence in a plant in an inducible or constitutive manner are provided, more particularly regulating expression of a PR-1 gene to enhance disease resistance in a plant. The methods comprise transforming a plant cell to comprise a heterologous nucleotide sequence or PR-1 gene operably linked to one of the inducible or constitutive promoters of the present invention and regenerating a stably transformed plant from the transformed plant cell. Transformed plants and seeds having enhanced disease resistance are also provided.</p> |           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |



## FAMILY OF MAIZE PR-1 GENES AND PROMOTERS

5

### FIELD OF THE INVENTION

The present invention relates to the field of plant molecular biology, more particularly to regulation of gene expression and enhancing disease resistance in plants.

### BACKGROUND OF THE INVENTION

Disease in plants is caused by biotic and abiotic causes. Biotic causes include fungi, viruses, bacteria, and nematodes. Of these, fungi are the most frequent causative agent of disease on plants. Abiotic causes of disease in plants include extremes of temperature, water, oxygen, soil pH, plus nutrient-element deficiencies and imbalances, excess heavy metals, and air pollution.

A host of cellular processes enables plants to defend themselves from disease caused by pathogenic agents. These processes apparently form an integrated set of resistance mechanisms that is activated by initial infection and then limits further spread of the invading pathogenic microorganism.

Subsequent to recognition of a potentially pathogenic microbe, plants can activate an array of biochemical responses. Generally, the plant responds by inducing several local responses in the cells immediately surrounding the infection site. The most common resistance response observed in both nonhost and race-specific interactions is termed the "hypersensitive response" (HR). In the hypersensitive response, cells contacted by the pathogen, and often neighboring cells, rapidly collapse and dry in a necrotic fleck. Other responses include the deposition of callose, the physical thickening of cell walls by

lignification, and the synthesis of various antibiotic small molecules and proteins, among which are the pathogenesis-related (PR) proteins. Genetic factors in both the host and the pathogen determine the specificity of these local responses, which can be very effective in limiting the spread of infection.

5 Pathogenesis-related proteins, which have been described in a number of plants (see Bowles (1990) *Ann. Rev. Biochem.* 59:873-907 for review), include the PR-1 proteins. Although their biochemical functions remain unknown, expression of PR-1 proteins is generally induced by pathogens and many abiotic treatments associated with the elicitation of the defense response, more particularly a  
10 hypersensitive response (see WO 89/02437 for a review). In tobacco, PR-1 protein expression is induced by viral infection and salicylic acid (SA) treatment (van Loon *et al.* (1987) *Plant Mol. Biol.* 9:593; Ward *et al.* (1991) *Plant Cell* 3:1085). Barley plants resistant to powdery mildew caused by *Erysiphe graminis* accumulate PRb-1 (a basic PR-1) mRNA 12 hours after inoculation with that  
15 pathogen, while susceptible plants do not, indicating these proteins serve as antipathogenic agents that contribute to disease resistance. Ethylene, jasmonic acid (JA), and SA also induce the accumulation of PRb-1 in the resistant cultivars, but not in related susceptible lines (Muradov *et al.* (1993) *Plant Mol. Biol.* 23:439). Salicylic acid induces PR-1 protein accumulation in maize leaves. Ultraviolet light  
20 and *C. carbonum* (tox-) inoculations induce protein accumulation in Pr (*hml*) leaves (in Crane *et al.* (1996), *Biology of Plant-Microbe Interactions* (International Society for Molecular Plant-Microbe Interactions), pp. 223-226). These observations make maize PR-1 genes and their promoters ideal candidates for use in the development of transgenic plants, particularly transgenic plants having  
25 enhanced disease resistance.

Thus, isolation and characterization of PR-1 genes and their corresponding promoters, which can serve as regulatory regions for expression of their native gene or other heterologous nucleotide sequences of interest, are needed for genetic  
30 manipulation of plants to exhibit specific phenotypic traits, particularly enhanced disease resistance, either in response to a given stimulus or in a constitutive manner.

### SUMMARY OF THE INVENTION

Compositions and methods for regulating gene expression in a plant are provided. Compositions are novel nucleotide sequences for inducible and  
5 constitutive plant promoters, more particularly promoters isolated from a family of maize genes encoding pathogenesis-related PR-1 proteins. Methods for regulating expression of a nucleotide sequence of interest in a plant using the promoter sequences disclosed herein are provided. The methods comprise transforming a plant cell with a nucleotide sequence of interest that is operably linked to one of  
10 the plant promoters of the present invention and regenerating a stably transformed plant from the transformed plant cell. Where the promoter is an inducible promoter of the invention, exposure of the transformed plant, or a particular tissue of the plant, to a stimulus activates, within the exposed tissues of the plant, expression of the nucleotide sequence operably linked to the particular inducible promoter  
15 disclosed herein.

Compositions of the invention also include the nucleotide sequences for novel maize PR-1 genes and the predicted amino acid sequences for the pathogenesis-related proteins encoded thereby. These nucleotide sequences are useful in the genetic manipulation of any plant when operably linked to a promoter  
20 that drives expression of a coding sequence in a plant cell, more particularly the PR-1 inducible and constitutive promoters disclosed herein. In this manner, transformed plants and progeny having increased resistance to pathogens and their related diseases may be obtained.

### 25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the plasmid vector PHP3953 comprising the GUS gene operably linked to the ubiquitin promoter. Promoter fragments of the present invention were recloned into this plasmid in place of the ubiquitin promoter, and the resulting plasmid DNA was available for use in transformation studies to test  
30 for promoter activity.

Figure 2 provides a vector for expression of the PR-1 genes of the invention.

### DETAILED DESCRIPTION OF THE INVENTION

Compositions of the present invention are nucleic acid molecules comprising novel nucleotide sequences for plant promoters for five maize genes, hereinafter designated as PR-1#52, PR-1#70, PR-1#81, PR-1#83, and PR-1#93, and the nucleotide sequences and respective predicted amino acid sequences for the pathogenesis-related class I (PR-1) proteins encoded by four of these maize genes. Nucleotide sequences for the promoters of these PR-1 genes are set forth in SEQ ID NOs: 1-5, respectively. Nucleotide sequences for the maize PR-1 genes PR-1#52, PR-1#70, PR-1#81, PR-1#83, and PR-1#93 are set forth in SEQ ID NOs: 6, 8, 10, 12, and 14, respectively. Amino acid sequences for the PR-1 proteins encoded by these genes are set forth in SEQ ID NOs: 7, 9, 11, 13, and 15, respectively. Four of these genes, PR-1#52, PR-1#70, PR-1#81, and PR-1#93 are novel. PR-1#83 is homologous to a known maize PR-1 gene, the cDNA sequence of which is published as GenBank Accession No. U82200. The PR-1 promoters of the invention were isolated from the 5p untranslated region flanking their respective transcription initiation sites. Methods for isolation of promoter regions are well known in the art. The specific method used to obtain the promoters of the present invention is described in Example 1 below.

In particular, the present invention provides for isolated nucleic acid molecules comprising nucleotide sequences for plant promoters shown in SEQ ID NOs: 1-5, nucleotide sequences encoding the amino acid sequences shown in SEQ ID NOs: 7, 9, 11, and 15, or the nucleotide sequences encoding the DNA sequence deposited in a bacterial host as ATCC Accession No. \_\_\_\_\_. Further provided are polypeptides having an amino acid sequence encoded by a nucleic acid molecule described herein, for example those set forth in SEQ ID NOs: 6, 8, 10, and 14, those deposited as ATCC Accession No. \_\_\_\_\_, and fragments and variants thereof.

Plasmids containing the PR-1 promoter and gene nucleotide sequences of the invention were deposited with American Type Culture Collection (ATCC), Manassas, Virginia, and assigned Accession No. \_\_\_\_\_. This deposit will be

maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. § 112.

5           Thus, the invention encompasses isolated or substantially purified nucleic acid or protein compositions. An "isolated" or "purified" nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically  
10 synthesized. Preferably, an "isolated" nucleic acid molecule is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid molecule (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain  
15 less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid molecule is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, 5%, (by dry weight) of contaminating protein. When  
20 the protein of the invention or biologically active portion thereof is recombinantly produced, preferably, culture medium represents less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

          The compositions of the invention include isolated nucleic acid molecules comprising the promoter nucleotide sequences set forth in SEQ ID NOs: 1-5. By  
25 "promoter" is intended a regulatory region of DNA usually comprising a TATA box capable of directing RNA polymerase II to initiate RNA synthesis at the appropriate transcription initiation site for a particular coding sequence. A promoter may additionally comprise other recognition sequences generally positioned upstream or 5p to the TATA box, referred to as upstream promoter  
30 elements, which influence the transcription initiation rate. It is recognized that having identified the nucleotide sequences for the promoter regions disclosed herein, it is within the state of the art to isolate and identify further regulatory

elements in the 5' untranslated region upstream from the particular promoter regions identified herein. Thus, for example, the promoter regions disclosed herein may further comprise upstream regulatory elements that confer tissue-specific expression of any heterologous nucleotide sequence operably linked to one of the disclosed promoter sequences. See particularly Australian Patent No. AU-A-77751/94 and U.S. Patent Nos. 5,466,785 and 5,635,618. Depending upon the promoter sequence utilized, the pattern of expression will be inducible, for example, with the promoter sequences disclosed in SEQ ID NOs: 1-4, or constitutive, for example, with the promoter sequence disclosed in SEQ ID NO: 5.

10       The maize inducible and constitutive promoter sequences of the present invention, when assembled within a DNA construct such that the promoter is operably linked to a heterologous nucleotide sequence of interest, enable expression of the heterologous nucleotide sequence in the cells of a plant stably transformed with this DNA construct. By "heterologous nucleotide sequence" is intended a sequence that is not naturally occurring with the promoter sequence. While this nucleotide sequence is heterologous to the promoter sequence, it may be homologous, or native, or heterologous, or foreign, to the plant host. Choice of the promoter sequence will determine when and where within the organism the heterologous nucleotide sequence is expressed. Thus, where continuous expression is desired throughout the cells of a plant, a constitutive promoter of the invention is utilized. In contrast, where gene expression in response to a stimulus is desired, an inducible promoter of the invention is the regulatory element of choice. When using an inducible promoter, expression of the heterologous nucleotide sequence is initiated in cells in response to a stimulus. By "stimulus" is intended a chemical, which may be applied externally or may accumulate in response to another external stimulus; a pathogen, which may, for example, induce expression as a result of invading a plant cell; or other factor such as environmental stresses, including but not limited to, drought, temperature, and salinity.

30       Compositions of the invention also include the nucleotide sequences for four maize PR-1 genes as set forth in SEQ ID NOs: 6, 8, 10, and 14 and the corresponding amino acid sequences for the PR-1 proteins encoded thereby as set forth in SEQ ID NOs: 7, 9, 11, and 15, respectively. These gene sequences may be

assembled into a DNA construct such that the gene is operably linked to a promoter that drives expression of a coding sequence in a plant cell. Plants stably transformed with this DNA construct express, either in a constitutive or inducible manner, a PR-1 protein of the invention. Expression of this protein creates or  
5 enhances disease resistance in the transformed plant.

Fragments and variants of these native nucleotide and amino acid sequences are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide or amino acid sequence. Fragments of a promoter nucleotide sequence may retain their regulatory activity. Thus, for example, less than the entire promoter  
10 sequences disclosed herein may be utilized to drive expression of an operably linked nucleotide sequence of interest, such as a nucleotide sequence encoding a heterologous protein. It is within skill in the art to determine whether such fragments decrease expression levels or alter the nature of expression, i.e., constitutive or inducible expression. Alternatively, fragments of a promoter nucleotide sequence  
15 that are useful as hybridization probes, such as described below, generally do not retain this regulatory activity.

Nucleic acid molecules that are fragments of a promoter nucleotide sequence comprise at least 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800 or 900 nucleotides, or up to  
20 the number of nucleotides present in the full-length promoter nucleotide sequence disclosed herein (i.e., 957, 1218, 969, 1149, or 255 for SEQ ID NO: 1, 2, 3, 4, or 5, respectively). Fragments of a promoter sequence that retain their regulatory activity comprise at least 30, 35, 40 contiguous nucleotides, preferably at least 50 contiguous nucleotides, more preferably at least 75 contiguous nucleotides, still  
25 more preferably at least 100 contiguous nucleotides of the particular promoter nucleotide sequence disclosed herein. Preferred fragment lengths depend upon the objective and will also vary depending upon the particular promoter sequence.

The nucleotides of such fragments will usually comprise the TATA  
30 recognition sequence of the particular promoter sequence. Such fragments may be obtained by use of restriction enzymes to cleave the naturally occurring

promoter nucleotide sequence disclosed herein; by synthesizing a nucleotide sequence from the naturally occurring sequence of the promoter DNA sequence; or may be obtained through the use of PCR technology. See particularly, Mullis *et al.* (1987) *Methods Enzymol.* 155:335-350, and Erlich, ed. (1989) *PCR Technology* (Stockton Press, New York). Variants of these promoter fragments, such as those resulting from site-directed mutagenesis, are also encompassed by the compositions of the present invention.

With respect to PR-1 gene nucleotide sequences (i.e., for example, the sequences set forth in SEQ ID NOs: 6, 8, 10, and 14), fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native PR-1 protein, i.e., the sequences set forth in SEQ ID NOs: 7, 9, 11, and 15, and hence enhance disease resistance when expressed in a plant. Alternatively, fragments of a coding nucleotide sequence that are useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the entire nucleotide sequence encoding the proteins of the invention.

A fragment of a PR-1 nucleotide sequence that encodes a biologically active portion of a PR-1 protein of the invention will encode at least 15, 25, 30, 40, 50, 75, 100, or 150 contiguous amino acids, or up to the total number of amino acids present in a full-length PR-1 protein of the invention (for example, 156, 231, 160, or 214 amino acids for SEQ ID NO: 7, 9, 11, or 15, respectively). Fragments of a PR-1 nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of a PR-1 protein.

A biologically active portion of a PR-1 protein can be prepared by isolating a portion of one of the PR-1 nucleotide sequences of the invention, expressing the encoded portion of the PR-1 protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the PR-1 protein. Nucleic acid molecules that are fragments of a PR-1 nucleotide sequence comprise at least 15, 20, 50, 75, 100, 325, 350, 375, 400, 425, 450,

500, 550, 600, 650, 700, or 800 nucleotides, or up to the number of nucleotides present in a full-length PR-1 nucleotide sequence disclosed herein (for example, 866, 973, 887, or 806 nucleotides for SEQ ID NO: 6, 8, 10, or 14, respectively).

5 By "variants" is intended sequences having substantial similarity with a promoter or gene nucleotide sequence disclosed herein. For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the PR-1 proteins of the invention. Naturally occurring allelic variants such as these  
10 can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a PR-1 protein of the invention.  
15 Generally, nucleotide sequence variants of the invention will have at least 40%, 50%, 60%, 70%, generally, 80%, preferably 85%, 90% to 95%, even 98% or more sequence identity to the respective native nucleotide sequence.

By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the  
20 N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Such variants may result from, for example, genetic polymorphism or from human manipulation.

25 The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the PR-1 proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in  
30 the art. See, for example, Kunkel, T. (1985) *Proc. Natl. Acad. Sci. USA*

82:488-492; Kunkel *et al.* (1987) *Methods in Enzymol.* 154:367-382; US Patent No. 4,873,192; Walker and Gaastra (eds.) *Techniques in Molecular Biology*, MacMillan Publishing Company, NY (1983) and the references cited therein.

Thus, the PR-1 promoter and gene nucleotide sequences of the invention  
5 include both the naturally occurring sequences as well as mutant forms.  
Likewise, the proteins of the invention encompass both naturally occurring  
proteins as well as variations and modified forms thereof. Such variants will  
continue to possess the desired PR-1 promoter or antipathogenic defense protein  
activity. Obviously, the mutations that will be made in the DNA encoding a  
10 variant protein must not place the sequence out of reading frame and preferably  
will not create complementary regions that could produce secondary mRNA  
structure. See, for example, EP Patent Application Publication No. 75,444.

In this manner, the present invention encompasses the PR-1 proteins as  
well as components and fragments thereof. That is, it is recognized that  
15 component polypeptides or fragments of the proteins may be produced which  
retain PR-1 protein activity that enhances disease resistance in a plant. These  
fragments include truncated sequences, as well as N-terminal, C-terminal,  
internal and internally deleted amino acid sequences of the proteins.

The deletions, insertions, and substitutions of the protein sequences  
20 encompassed herein are not expected to produce radical changes in the  
characteristics of the PR-1 proteins. However, when it is difficult to predict the  
exact effect of the substitution, deletion, or insertion in advance of doing so, one  
skilled in the art will appreciate that the effect will be evaluated by routine  
screening assays. That is, the activity of the modified protein sequences can be  
25 evaluated by monitoring of the plant defense system. See, for example U.S.  
Patent No. 5,614,395, herein incorporated by reference.

Thus the nucleotide sequences for the PR-1 promoters and genes of the  
present invention include the native forms as well as fragments and variants  
thereof. Similarly, the PR-1 proteins of the invention include the native forms as  
30 well as fragments and variants thereof. The variant nucleotide sequences and  
variant proteins will share substantial homology with their naturally occurring

sequences. By "substantial homology" is intended a sequence exhibiting substantial functional and structural equivalence with the native or naturally occurring sequence. Any functional or structural differences between substantially homologous sequences do not effect the ability of the sequence to function as a promoter or as a PR-1 protein as disclosed in the present invention. Thus, for example, any sequence having substantial sequence homology with the sequence of a particular inducible promoter of the present invention will direct expression of an operably linked heterologous nucleotide sequence in response to a stimulus. Two nucleotide sequences or polypeptides are considered substantially homologous when they have at least about 50%, 60%, to 70%, generally at least about 80%, preferably at least about 85%, 90%, to 98% sequence homology. Substantially homologous sequences of the present invention include variants of the disclosed sequences such as those that result from site-directed mutagenesis, as well as synthetically derived sequences.

The nucleotide sequences of the invention can be used to isolate other homologous sequences in other plant species. Methods are readily available in the art for the hybridization of nucleic acid sequences. Promoter and coding sequences from other plants may be isolated according to well-known techniques based on their sequence homology to the PR-1 promoter and gene sequences set forth herein. In these techniques all or part of the known promoter or coding sequence is used as a probe which selectively hybridizes to other PR-1 promoter and gene sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism.

For example, the entire PR-1 promoter or PR-1 gene sequence or portions thereof may be used as probes capable of specifically hybridizing to corresponding PR-1 promoter or PR-1 coding sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such probes may be used to amplify the PR-1 promoter or coding sequences of interest from a chosen organism by the well-known process of polymerase chain reaction

(PCR). This technique may be used to isolate additional promoter or coding sequences from a desired organism or as a diagnostic assay to determine the presence of PR-1 promoter or coding sequences in an organism.

Such techniques include hybridization screening of plated DNA libraries  
5 (either plaques or colonies; see, e.g., Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York) and amplification by PCR using oligonucleotide primers corresponding to sequence domains conserved among the amino acid sequences (see, e.g., Innis *et al.*, eds. (1990) *PCR Protocols, a Guide to Methods and*  
10 *Applications* (Academic Press, New York).

For example, hybridization of such sequences may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented  
15 by a wash stringency of 40-45% Formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively), to DNA encoding the PR-1 genes disclosed herein in a standard hybridization assay. See Sambrook *et al.* (1989) *Molecular Cloning: A*  
20 *Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). In general, promoter sequences and sequences that code for the PR-1 proteins of the invention and hybridize to the sequences disclosed herein will be at least 40% to 50% homologous, 60% to 70% homologous, and even 85%, 90% to 98% homologous or more with the disclosed sequence. That is, the sequence  
25 similarity of sequences may range, sharing at least about 40% to 50%, about 60% to 70%, and even at least about 80%, 85%, 90%, 95% to 98% sequence similarity.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence",

(b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; by the homology alignment algorithm of Needleman *et al.* (1970) *J. Mol. Biol.* 48:443; by the search for similarity method of Pearson *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:2444; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California; GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins *et al.* (1988) *Gene* 73:237-244 (1988); Higgins *et al.* (1989) *CABIOS* 5:151-153 ; Corpet *et al.* (1988) *Nucleic Acids Research* 16:10881-90; Huang *et al.* (1992) *Computer Applications in the Biosciences* 8:155-65, and Person *et al.* (1994) *Methods of Molecular Biology* 24:307-331;

preferred computer alignment methods also include the BLASTP, BLASTN, and BLASTX algorithms (see Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410).

Alignment is also often performed by inspection and manual alignment.

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue

occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

5           (e)(i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will  
10 recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning, and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%,  
15 80%, 90%, and most preferably at least 95%. Polypeptides, which are "substantially similar" share sequences as, noted above except that residue positions, which are not identical, may differ by conservative amino acid changes.

Another indication that nucleotide sequences are substantially identical is  
20 if two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C to about 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.  
25 Typically, stringent wash conditions are those in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least about 50, 55, or 60°C. However, nucleic acids, which do not hybridize to each other under stringent conditions, are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid  
30 is created using the maximum codon degeneracy permitted by the genetic code.

One indication that two nucleic acid sequences are substantially identical is that the polypeptide, which the first nucleic acid encodes, is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

- (e)(ii) The term "substantial identity" in the context of a peptide indicates
- 5 that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman *et al.* (1970) *J. Mol. Biol.* 48:443.
- 10 An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution.

- The PR-1 proteins described herein may be used alone or in combination
- 15 with other proteins or agents to protect against plant diseases and pathogens. Other plant defense proteins include those described in the copending applications both entitled "Methods for Enhancing Disease Resistance in Plants", U.S. Application Serial No. 60/076,151, filed February 26, 1998, and U.S. Application Serial No. 60/092,464, filed July 11, 1998, both of which are herein
- 20 incorporated by reference.

- The PR-1 promoter and gene nucleotide sequences disclosed herein are useful for genetic engineering of plants to express a phenotype of interest. The promoter sequences may be used to drive expression of heterologous nucleotide sequences not naturally occurring with the particular promoter sequence.
- 25 Alternatively, the promoter sequences may be used to drive expression of their native, i.e., naturally occurring, PR-1 gene sequences disclosed herein. The PR-1 gene sequences, when operably linked with a promoter that drives expression in a plant cell, may be used to create or enhance disease resistance in a transformed plant.

- 30 More particularly, the nucleotide sequences for the inducible and constitutive PR-1 family of promoters disclosed in the present invention, as well as

variants and fragments thereof, are useful in the genetic manipulation of any plant when assembled within a DNA construct such that the promoter sequence is operably linked with a heterologous nucleotide sequence of interest. By "operably linked" is intended the transcription or translation of the heterologous nucleotide sequence is under the influence of the promoter sequence. In this manner, the nucleotide sequences for the promoters of the invention are provided in expression cassettes along with heterologous nucleotide sequences for expression in the plant of interest.

It is recognized that the promoter sequences of the invention may also be used with their native PR-1 coding sequences to genetically engineer plants having enhanced disease resistance. A DNA construct comprising the PR-1 promoter operably linked with its native PR-1 gene may be used to transform any plant of interest to bring about a change in phenotype. Where the promoter and its native gene is naturally occurring within a plant, i.e., in maize, transformation of the plant with these operably linked sequences results in a change in phenotype, such as enhanced disease resistance, or insertion of these operably linked sequences within a different region of the chromosomes thereby altering the plant's genome.

The promoters for the PR-1 genes designated PR-1#52, PR-1#70, PR-1#81, and PR-1#83 regulate expression of an operably linked nucleotide sequence in an inducible manner. That is, expression of the operably linked nucleotide sequence in a plant cell is induced in response to a stimulus, for example in response to pathogen invasion, externally applied chemicals, or environmental stresses.

In contrast, the promoter for the PR-1 gene designated PR-1#93 is a constitutive promoter, more particularly a weak constitutive promoter. By "constitutive" is intended expression in the cells throughout a plant at most times and in most tissues. Generally, by "weak" promoter is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Conversely, a strong promoter drives expression of a coding sequence at a high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

Thus, in one embodiment of the invention, expression cassettes will comprise a transcriptional initiation region comprising one of the promoter nucleotide sequences disclosed herein, or variants or fragments thereof, operably linked to the heterologous nucleotide sequence whose expression is to be  
5 controlled by the inducible or constitutive promoters of the invention. Such an expression cassette is provided with a plurality of restriction sites for insertion of the nucleotide sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The transcriptional cassette will include in the 5p-to-3p direction of  
10 transcription, a transcriptional and translational initiation region, a heterologous nucleotide sequence of interest, and a transcriptional and translational termination region functional in plants. The termination region may be native with the transcriptional initiation region comprising one of the promoter nucleotide sequences of the present invention, may be native with the DNA sequence of  
15 interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272;  
20 Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* 1989) *Nucleic Acids Res.* 17:7891-7903; Joshi *et al.* (1987) *Nucleic Acid Res.* 15:9627-9639.

The expression cassette comprising a promoter sequence of the present invention operably linked to a heterologous nucleotide sequence may also contain at least one additional nucleotide sequence for a gene to be cotransformed into the  
25 organism. Alternatively, the additional sequence(s) can be provided on another expression cassette.

Where appropriate, the heterologous nucleotide sequence whose expression is to be under the control of the promoter sequence of the present invention and any additional nucleotide sequence(s) may be optimized for increased expression  
30 in the transformed plant. That is, these nucleotide sequences can be synthesized using plant-preferred codons for improved expression. Methods are available in the art for synthesizing plant-preferred nucleotide sequences. See, for example,

U.S. Patent Nos. 5,380,831 and 5,436,391, and Murray et al. (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious  
5 polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the heterologous nucleotide sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified  
10 to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5p leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5p noncoding region)  
15 (Elroy-Stein *et al.* (1989) *Proc. Nat. Acad. Sci. USA* 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986)); MDMV leader (Maize Dwarf Mosaic Virus) (*Virology* 154:9-20); human immunoglobulin heavy-chain binding protein (BiP) (Macejak and Sarnow (1991) *Nature* 353:90-94); untranslated leader from the coat protein mRNA of alfalfa  
20 mosaic virus (AMV RNA 4) (Jobling and Gehrke (1987) *Nature* 325:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) *Molecular Biology of RNA*, pages 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology* 81:382-385). See also Della-Cioppa *et al.* (1987) *Plant Physiology* 84:965-968. Other methods known to enhance translation and/or  
25 mRNA stability can also be utilized, for example, introns, and the like.

In those instances where it is desirable to have the expressed product of the heterologous nucleotide sequence directed to a particular organelle, such as the chloroplast or mitochondrion, or secreted at the cell=s surface or extracellularly, the expression cassette may further comprise a coding sequence for a transit  
30 peptide. Such transit peptides are well known in the art and include, but are not limited to, the transit peptide for the acyl carrier protein, the small subunit of RUBISCO, plant EPSP synthase, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, for example, transitions and transversions, may be involved.

The expression cassette comprising the particular promoter sequence of the present invention operably linked to a heterologous nucleotide sequence of interest can be used to transform any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained. Transformation protocols may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of transforming plant cells include microinjection (Crossway *et al.* (1986) *Biotechniques* 4:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606), *Agrobacterium*-mediated transformation (Hinchey *et al.* (1988) *Biotechnology* 6:915-921), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford *et al.* U.S. Patent 4,945,050; Tomes *et al.* (1995) in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe *et al.* (1988) *Biotechnology* 6:923-926). Also see Weissinger *et al.* (1988) *Annual Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Datta *et al.* (1990) *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); Klein *et al.* (1988) *Plant Physiol.* 91:440-444 (maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839; Hooykaas-Van Slogteren and Hooykaas (1984) *Nature (London)* 311:763-764; Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of Ovule*

*Tissues*, ed. G. P. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports* 9:415-418; and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D=Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having inducible expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that inducible or constitutive expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved.

The PR-1 inducible and constitutive promoter nucleotide sequences and methods disclosed herein are useful in regulating expression of any heterologous nucleotide sequence in a host plant in order to vary the phenotype of a plant. Depending upon the PR-1 promoter used, expression may be regulated in an inducible or constitutive manner as previously described. Various changes in phenotype are of interest including modifying the fatty acid composition in a plant, altering the amino acid content of a plant, altering a plant's pathogen defense mechanism, and the like. These results can be achieved by providing expression of heterologous products or increased expression of endogenous products in plants. Alternatively, the results can be achieved by providing for a reduction of expression of one or more endogenous products, particularly enzymes or cofactors in the plant. These changes result in a change in phenotype of the transformed plant.

Genes of interest are reflective of the commercial markets and interests of those involved in the development of the crop. Crops and markets of interest

change, and as developing nations open up world markets, new crops and technologies will emerge also. In addition, as our understanding of agronomic traits and characteristics such as yield and heterosis increase, the choice of genes for transformation will change accordingly. General categories of genes of interest include for example, those genes involved in information, such as zinc fingers, those involved in communication, such as kinases, and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include genes encoding important traits for agronomics, insect resistance, disease resistance, herbicide resistance, sterility, grain characteristics, and commercial products. Genes of interest include, generally, those involved in oil, starch, carbohydrate, or nutrient metabolism as well as those affecting kernel size, sucrose loading, and the like.

Agronomically important traits such as oil, starch, and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing levels of lysine and sulfur, providing essential amino acids, and also modification of starch. Hordothionin protein modifications are described in U.S. Patent Application Serial Nos. 08/838,763, filed April 10, 1997; 08/824,379, filed March 26, 1997; 08/824,382, filed March 26, 1997; and U.S. Patent No. 5,703,409; herein incorporated by reference. Another example is lysine and/or sulfur rich seed protein encoded by the soybean 2S albumin described in U.S. Serial No. 08/618,911, filed March 20, 1996, and the chymotrypsin inhibitor from barley, Williamson *et al.* (1987) *Eur. J. Biochem.* 165:99-106, the disclosures of which are herein incorporated by reference.

Derivatives of the coding sequences can be made by site-directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide (BHL) is derived from barley chymotrypsin inhibitor, U.S. Serial No. 08/740,682 filed November 1, 1996, and PCT/US97/20441, filed October 31, 1997, the disclosures of each are incorporated herein by reference. Other

proteins include methionine-rich plant proteins such as from sunflower seed (Lilley *et al.* (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, ed. Applewhite (American Oil Chemists Society, Champaign, Illinois), pp. 497-502; herein incorporated by reference)); corn (Pedersen *et al.* (1986) *J. Biol. Chem.* 261:6279; Kirihara *et al.* (1988) *Gene* 71:359; both of which are herein incorporated by reference); and rice (Musumura *et al.* (1989) *Plant Mol. Biol.* 12:123, herein incorporated by reference). Other agronomically important genes encode latex, Floury 2, growth factors, seed storage factors, and transcription factors.

10 Insect resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for example *Bacillus thuringiensis* toxic protein genes (U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser *et al.* (1986) *Gene* 48:109); lectins (Van Damme *et al.* (1994) *Plant Mol. Biol.* 15 24:825); and the like.

Genes encoding disease resistance traits include detoxification genes, such as against fumonosin (U.S. Patent Application Serial No. 08/484,815, filed June 7, 1995); avirulence (avr) and disease resistance (R) genes (Jones *et al.* (1994) *Science* 266:789; Martin *et al.* (1993) *Science* 262:1432; Mindrinos *et al.* (1994) 20 *Cell* 78:1089); and the like.

Herbicide resistance traits may include genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance, in particular the S4 and/or 25 Hra mutations), genes coding for resistance to herbicides that act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the bar gene), or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide 30 chlorsulfuron.

Sterility genes can also be encoded in an expression cassette and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in U.S. Patent No. 5,583,210. Other genes include kinases  
5 and those encoding compounds toxic to either male or female gametophytic development.

The quality of grain is reflected in traits such as levels and types of oils, saturated and unsaturated, quality and quantity of essential amino acids, and levels of cellulose. In corn, modified hordothionin proteins, described in U.S.  
10 Patent Application Serial Nos. 08/838,763, filed April 10, 1997; 08/824,379, filed March 26, 1997; 08/824,382, filed March 26, 1997; and U.S. Patent No. 5,703,409 issued December 30, 1997, provide descriptions of modifications of proteins for desired purposes.

15 Commercial traits can also be encoded on a gene or genes that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in U.S. Patent No. 5,602,321 issued February 11, 1997. Genes such as B-Ketothiolase, PHBase  
20 (polyhydroxybutyrate synthase) and acetoacetyl-CoA reductase (see Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhyroxyalkanoates (PHAs).

Exogenous products include plant enzymes and products as well as those  
25 from other sources including procaryotes and other eukaryotes. Such products include enzymes, cofactors, hormones, and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.

30 Thus, the heterologous nucleotide sequence operably linked to one of the inducible promoters disclosed herein may be a structural gene encoding a protein

of interest. Examples of such heterologous genes include, but are not limited to, genes encoding proteins conferring resistance to abiotic stress, such as drought, temperature, salinity, and toxins such as pesticides and herbicides, or to biotic stress, such as attacks by fungi, viruses, bacteria, insects, and nematodes, and  
5 development of diseases associated with these organisms.

More particularly, the constitutive and inducible promoters disclosed herein are useful in transforming plants to express an avirulence gene either constitutively or in response to pathogen invasion as disclosed in the copending applications both entitled "Methods for Enhancing Disease Resistance in Plants", U.S. Application  
10 Serial No. 60/076,151, filed February 26, 1998, and U.S. Application Serial No. 60/092,464, filed July 11, 1998, both of which are herein incorporated by reference. When the constitutive promoter of the invention is used, the plant defense system is activated short of hypersensitive cell death. Thus, there is activation of the plant defense system at levels sufficient to protect from pathogen  
15 invasion. Alternatively, an inducible promoter may be used to induce the hypersensitive response. When used in this manner, induced expression of an avirulence gene causes activation of the hypersensitive plant defense system wherein the plant produces increased levels of antipathogenic factors such as PR proteins, i.e., PR-1, chitinases,  $\beta$ -glucanases, etc.; secondary metabolites;  
20 phytoalexins; reactive oxygen species; and the like. This hypersensitive response contains pathogen invasion, thereby enhancing disease resistance.

Alternatively, the heterologous nucleotide sequence operably linked to one of the PR-1 inducible or constitutive promoters disclosed herein may be an antisense sequence for a targeted gene. By "antisense DNA nucleotide sequence"  
25 is intended a sequence that is in inverse orientation to the 5p to 3p normal orientation of that nucleotide sequence. When delivered into a plant cell, expression of the antisense DNA sequence prevents normal expression of the DNA nucleotide sequence for the targeted gene. The antisense nucleotide sequence encodes an RNA transcript that is complementary to and capable of hybridizing to  
30 the endogenous messenger RNA (mRNA) produced by transcription of the DNA nucleotide sequence for the targeted gene. In this case, production of the native

protein encoded by the targeted gene is inhibited to achieve a desired phenotypic response. Thus the promoter sequences disclosed herein may be operably linked to antisense DNA sequences to reduce or inhibit expression of a native protein in the plant. Where the promoter operably linked to the antisense sequence is one of the  
5 inducible promoters of the invention, expression of a native protein is regulated in an antisense manner in response to a particular stimulus.

The nucleotide sequences for the PR-1 genes of the present invention, and variants and fragments thereof, are useful in the genetic manipulation of any plant when assembled in a DNA construct such that the gene sequence is operably linked  
10 to a promoter that drives expression of a coding sequence in a plant cell. Such a DNA construct can be used with transformation techniques, such as those described above, to create disease resistance in susceptible plant phenotypes or to enhance disease resistance in resistant plant phenotypes. Accordingly, the invention encompasses methods that are directed to protecting plants against  
15 fungal pathogens, viruses, nematodes, insects and the like.

By "disease resistance" is intended that the plants avoid the disease symptoms that are the outcome of plant-pathogen interactions. That is, pathogens are prevented from causing plant diseases and the associated disease symptoms. The method of transformation with a PR-1 gene of the invention can  
20 be utilized to protect plants from disease, particularly those diseases that are caused by plant pathogens.

Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, insects, nematodes, fungi, and the like. Viruses include tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf  
25 mosaic virus, etc. Specific fungal and viral pathogens for the major crops include: Soybeans: *Phytophthora megasperma* fsp. *glycinea*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae* (*Phomopsis sojae*), *Diaporthe phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora sojae*,  
30 *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotrichum truncatum*), *Corynespora cassiicola*, *Septoria glycines*, *Phyllosticta sojicola*, *Alternaria*

- alternata*, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*, *Phialophora gregata*, Soybean mosaic virus, *Glomerella glycines*, Tobacco Ring spot virus, Tobacco Streak virus, *Phakopsora pachyrhizi*, *Pythium aphanidermatum*, *Pythium*
- 5 *ultimum*, *Pythium debaryanum*, Tomato spotted wilt virus, *Heterodera glycines* *Fusarium solani*; Canola: *Albugo candida*, *Alternaria brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Mycosphaerella brassicicola*, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium roseum*, *Alternaria alternata*; Alfalfa: *Clavibacter michiganense* subsp. *insidiosum*,
- 10 *Pythium ultimum*, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*, *Pythium aphanidermatum*, *Phytophthora megasperma*, *Peronospora trifoliorum*, *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Leptotrochila medicaginis*, *Fusarium atrum*, *Xanthomonas campestris* p.v. *alfalfae*, *Aphanomyces euteiches*, *Stemphylium herbarum*, *Stemphylium*
- 15 *alfalfae*; Wheat: *Pseudomonas syringae* p.v. *atrofaciens*, *Urocystis agropyri*, *Xanthomonas campestris* p.v. *translucens*, *Pseudomonas syringae* p.v. *syringae*, *Alternaria alternata*, *Cladosporium herbarum*, *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Ustilago tritici*, *Ascochyta tritici*, *Cephalosporium gramineum*, *Collotetrachum graminicola*, *Erysiphe graminis*
- 20 f.sp. *tritici*, *Puccinia graminis* f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, *Puccinia striiformis*, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*, *Septoria avenae*, *Pseudocercospora herpotrichoides*, *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris*
- 25 *sorokiniana*, Barley Yellow Dwarf Virus, Brome Mosaic Virus, Soil Borne Wheat Mosaic Virus, Wheat Streak Mosaic Virus, Wheat Spindle Streak Virus, American Wheat Striate Virus, *Claviceps purpurea*, *Tilletia tritici*, *Tilletia laevis*, *Ustilago tritici*, *Tilletia indica*, *Rhizoctonia solani*, *Pythium arrhenomanes*, *Pythium graminicola*, *Pythium aphanidermatum*, High Plains Virus, European
- 30 wheat striate virus; Sunflower: *Plasmophora halstedii*, *Sclerotinia sclerotiorum*,

- Aster Yellows, *Septoria helianthi*, *Phomopsis helianthi*, *Alternaria helianthi*,  
*Alternaria zinniae*, *Botrytis cinerea*, *Phoma macdonaldii*, *Macrophomina*  
*phaseolina*, *Erysiphe cichoracearum*, *Rhizopus oryzae*, *Rhizopus arrhizus*,  
*Rhizopus stolonifer*, *Puccinia helianthi*, *Verticillium dahliae*, *Erwinia*  
5 *carotovorum* pv. *carotovora*, *Cephalosporium acremonium*, *Phytophthora*  
*cryptogea*, *Albugo tragopogonis*; Corn: *Fusarium moniliforme* var. *subglutinans*,  
*Erwinia stewartii*, *Fusarium moniliforme*, *Gibberella zae* (*Fusarium*  
*graminearum*), *Stenocarpella maydi* (*Diplodia maydis*), *Pythium irregulare*,  
*Pythium debaryanum*, *Pythium graminicola*, *Pythium splendens*, *Pythium*  
10 *ultimum*, *Pythium aphanidermatum*, *Aspergillus flavus*, *Bipolaris maydis* O, T  
(*Cochliobolus heterostrophus*), *Helminthosporium carbonum* I, II & III  
(*Cochliobolus carbonum*), *Exserohilum turcicum* I, II & III, *Helminthosporium*  
*pedicellatum*, *Physoderma maydis*, *Phyllosticta maydis*, *Kabatie-maydis*,  
*Cercospora sorghi*, *Ustilago maydis*, *Puccinia sorghi*, *Puccinia polysora*,  
15 *Macrophomina phaseolina*, *Penicillium oxalicum*, *Nigrospora oryzae*,  
*Cladosporium herbarum*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia*  
*pallescent*, *Clavibacter michiganense* subsp. *nebraskense*, *Trichoderma viride*,  
Maize Dwarf Mosaic Virus A & B, Wheat Streak Mosaic Virus, Maize Chlorotic  
Dwarf Virus, *Claviceps sorghi*, *Pseudomonas avenae*, *Erwinia chrysanthemi* pv.  
20 *zea*, *Erwinia carotovora*, *Cornstunt Spiroplasma*, *Diplodia macrospora*,  
*Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora*  
*philippinensis*, *Peronosclerospora maydis*, *Peronosclerospora sacchari*,  
*Spacelotheca reiliana*, *Physopella zae*, *Cephalosporium maydis*,  
*Cephalosporium acremonium*, Maize Chlorotic Mottle Virus, High Plains Virus,  
25 Maize Mosaic Virus, Maize Rayado Fino Virus, Maize Streak Virus, Maize  
Stripe Virus, Maize Rough Dwarf Virus; Sorghum: *Exserohilum turcicum*,  
*Colletotrichum graminicola* (*Glomerella graminicola*), *Cercospora sorghi*,  
*Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v.  
*syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*,  
30 *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*, *Fusarium*

*moniliforme*, *Alternaria alternate*, *Bipolaris sorghicola*, *Helminthosporium sorghicola*, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas avenae* (*Pseudomonas alboprecipitans*), *Ramulispora sorghi*, *Ramulispora sorghicola*, *Phyllachara sacchari*, *Sporisorium reilianum* (*Sphacelotheca reiliana*),  
 5 *Sphacelotheca cruenta*, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*,  
*Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium*  
 10 *graminicola*, etc.

Nematodes include parasitic nematodes such as root knot, cyst and lesion nematodes, etc.

Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera,  
 15 Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Coleoptera and Lepidoptera. Insect pests of the invention for the major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus*  
 20 *lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*, sugarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*, northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus spp.*, wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white  
 25 grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot;  
 30 *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscurus*, grass thrips;

*Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted spider mite;  
Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall  
 armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser  
 cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*,  
 5 white grub; *Eleodes*, *Conoderus*, and *Aeolus spp.*, wireworms; *Oulema*  
*melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle;  
*Sphenophorus maidis*, maize billbug; *Rhopalosiphum maidis*; corn leaf aphid;  
*Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch  
 bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine  
 10 spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia*  
*unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus*  
*lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western cutworm;  
*Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf  
 beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata*  
 15 *howardi*, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*,  
 greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*,  
 redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper;  
*Melanoplus sanguinipes*, migratory grasshopper; *Mayetiola destructor*, Hessian  
 fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem  
 20 maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips;  
*Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower:  
*Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower  
 moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot  
 beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis*  
 25 *virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera*  
*exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus*  
*grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*,  
 cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus*  
*lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper;  
 30 *Melanoplus differentialis*, differential grasshopper; *Thrips tabaci*, onion thrips;

*Frankliniella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatilis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestanii*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* spp., Root maggots.

In this manner, the nucleotide sequences for the PR-1 genes are provided in expression cassettes as previously described to provide for expression in a plant of interest. Expression of the disclosed PR-1 gene sequences may be driven by any promoter that is operable within a plant cell, with the preferred promoter depending upon the desired outcome. Generally, it will be beneficial to regulate expression of the PR-1 gene using an inducible promoter, particularly a pathogen-inducible promoter such as the PR-1 inducible promoters disclosed

herein. Thus any plant of interest may be transformed with an expression cassette comprising a PR-1 gene sequence disclosed herein operably linked with one of the PR-1 promoters disclosed herein, including the native PR-1 promoter for that PR-1 gene. Where expression of a PR-1 gene is driven by its native promoter,  
5 transformation of a plant with these operably linked sequences results in a plant phenotype exhibiting enhanced disease resistance. Where the transformed plant is the native plant from which the PR-1 promoter and gene sequences have been isolated, i.e., maize, the transformed plant will exhibit an altered phenotype, or have the operably linked sequences inserted at a new location within its genome.

10 Other inducible promoters suitable for driving expression of the PR-1 gene sequences disclosed herein include the promoters regulating expression of other pathogenesis-related proteins that are induced following infection by a pathogen; e.g., other PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol.* 89:245-254;  
15 Uknes *et al.* (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116.

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau *et al.* (1987) *Plant Mol. Biol.* 1:335-342; Matton *et al.* (1989) *Molecular Plant-Microbe Interactions* 2:325-  
20 331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch *et al.* (1988) *Molecular and General Genetics* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen *et al.* (1996) *Plant J.* 10:955-966; Zhang *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner *et al.* (1993) *Plant J.* 3:191-201; Siebertz *et al.* (1989) *Plant Cell* 1:961-  
25 968; and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiological and Molecular Plant Pathology* 41:189-200).

Additionally, as pathogens find entry into plants through wounds or insect  
30 damage, a wound inducible promoter may be used in the DNA constructs of the

invention. Such wound inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan, *Ann. Rev. Phytopath.* 28:425-449; Duan *et al.*, *Nature Biotechnology* 14:494-498); wun1 and wun2, U.S. Patent No. 5,428,148; win1 and win2 (Stanford *et al.* *Mol Gen Genet* 215:200-208); systemin (McGurl *et al.* *Science* 225:1570-1573); WIP1 (Rohmeier *et al.* *Plant Mol. Biol.* 22:783-792; Eckelkamp *et al.* *FEBS Letters* 323:73-76); MPI gene (Corderok *et al.* *Plant Journal* 6(2):141-150); and the like, herein incorporated by reference.

Alternatively, constitutive promoters can be utilized to provide continuous expression of the antipathogenic PR-1 proteins disclosed herein. Such constitutive promoters include the PR-1 promoter disclosed herein (PR-1#93, SEQ ID NO: 5), 35S promoter, ubiquitin promoter, and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142. See also copending U.S. Application Serial No. 60/076,075 entitled "Constitutive Maize Promoters," filed February 26, 1998, herein incorporated by reference.

Tissue-specific promoters can be utilized to target enhanced disease resistance within a particular plant tissue. Tissue-specific promoters include Yamamoto *et al.* (1997) *Plant J.* 12(2):255-265; Kawamata *et al.* (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen *et al.* (1997) *Mol Gen Genet.* 254(3):337-343; Russell *et al.* (1997) *Transgenic Res.* 6(2):157-168; Rinehart *et al.* (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp *et al.* (1996) *Plant Physiol.* 112(2):525-535; Canevascini *et al.* (1996) *Plant Physiol.* 112(2):513-524; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35(5):773-778; Lam (1994) *Results Probl Cell Differ.* 20:181-196; Orozco *et al.* (1993) *Plant Mol Biol.* 23(6):1129-1138; Matsuoka *et al.* (1993) *Proc Natl. Acad. Sci. USA* 90(20):9586-9590; and Guevara-Garcia *et al.* (1993) *Plant J.* 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

The expression cassette comprising a desired promoter operably linked to a nucleotide sequence for the PR-1 genes of the present invention can be used to transform any plant as described elsewhere herein. In this manner, one can obtain genetically modified plants, plant cells, plant tissue, seed, and the like that have

enhanced disease resistance to a pathogen.

The following examples are offered by way of illustration and not by way of limitation.

5

## EXPERIMENTAL

Promoter regions for five maize genes encoding pathogenesis-related class  
10 1 (PR-1) proteins designated as PR-1#52, PR-1#70, PR-1#81, PR-1#83, and PR-  
1#93 were isolated from maize plants and cloned. The promoter sequences for  
these five maize PR-1 genes are set forth in SEQ ID NOs: 1, 2, 3, 4, and 5,  
respectively. These genes were selected as sources of inducible promoters based on  
the developmental and spatial expression of their gene products. The method for  
15 promoter isolation is described below.

### Example 1: Isolation of Promoter Sequences

The procedure for promoter isolation is described in the User Manual for  
20 the Genome Walker kit sold by Clontech Laboratories, Inc., Palo Alto, California.  
Genomic DNA from maize line A63 was prepared by grinding 10-day-old seedling  
leaves in liquid nitrogen, and the DNA prepared as described by Chen and  
Dellaporta (1994) in *The Maize Handbook*, ed. Freeling and Walbot (Springer-  
Verlag, Berlin). RNase A was added to 10  $\Phi$ g/ml and then incubated at 37C for 1  
25 hr. The DNA was then extracted once with phenol-chloroform, then chloroform,  
then ethanol precipitated and resuspended in TE (10 mM Tris pH 8.0, 1 mM  
EDTA). The DNA was then used exactly as described in the Genome Walker User  
Manual (Clontech PT3042-1 version PR68687). Briefly, the DNA was digested  
separately with restriction enzymes DraI, EcoRV, PvuII, ScaI, and StuI, all blunt-  
30 end cutters. The DNA was extracted with phenol, then chloroform, then ethanol  
precipitated. The Genome Walker adapters were ligated onto the ends of the  
restricted DNA. The resulting DNA is referred to as DL1-DL5, respectively.

For isolation of specific promoter regions, two nonoverlapping gene-specific primers (27-30 bp in length) were designed from the 5p end of 5 maize PR-1 cDNAs identified from sequence databases. The primers were designed to amplify the region upstream of the coding sequence, i.e., the 5p untranslated region and promoter of the chosen gene. The sequences of the primers are given below for each promoter described. The first round of PCR was performed on each DNA sample (DL1-5) with Clontech primer AP1 (sequence 5p-gtaatacgcactcactatagggc-3p; SEQ ID NO: 16) and the gene-specific primer (gsp)1 with the following sequences:

PR-1#52:

gspl (DO 18914): 5p-agc taa aca cac gac gac cct agt aga cga-3p (SEQ ID NO: 17)

PR-1#70:

gspl (DO 20540): 5p-ctc cca cct cgg cgc ccg cca tta cag-3p (SEQ ID NO: 18)

PR-1#81:

gspl (DO 20770): 5p-gcc agt cca tca cgg cgg cgc gga aca g-3p (SEQ ID NO: 19)

PR-1#83:

gspl (DO 18912): 5p-cac gct agc ctc ggt gcc att gtt tgt tgt-3p (SEQ ID NO: 20)

PR-1#93:

gspl (DO 18123): 5p-ggc tag acg act agt agg ctt cgg aaa c-3p (SEQ ID NO: 21)

PCR was performed in a model PTC-100 thermal cycler with HotBonnet from MJ Research (Watertown, Maine) using reagents supplied with the Genome Walker kit. The following cycle parameters were used: seven cycles of 94C for 2 sec, then 72C for 3 min, followed by 32 cycles of 94C for 2 sec, and 67C for 3 min. Finally, the samples were held at 67C for 4 min, then at 4C until further

analysis.

As described in the User Manual, the DNA from the first round of PCR was then diluted and used as a template in a second round of PCR using the Clontech AP2 primer (sequence 5p-actatagggcacgcgtggt-3p; SEQ ID NO: 22) and  
5 gene-specific primer (gsp)2 with the following sequences:

PR-1#52:

gsp2 (DO 18915): 5p-gca cgg cat cac ggc gac cat ggc caa-3p (SEQ ID NO: 23)

10 PR-1#70:

gsp2 (DO 20352): 5p-gcg ccc gcc att aca gcc tcc tcg cgt ctt-3p (SEQ ID NO: 24)

PR-1#81:

15 gsp2 (DO 20539): 5p-ggg cta gct gaa ggc tga agc aga tgg-3p (SEQ ID NO: 25)

PR-1#83:

gsp2 (DO 18913): 5p-gac gat ggc tgc cat ggc cac agc tag gag-3p (SEQ ID NO: 26)

20

PR-1#93:

gsp2 (DO 18911): 5p-cca tgg ccg cag cga ggg cag cta gcg-3p (SEQ ID NO: 27)

The cycle parameters for the second round were: 5 cycles of 94C for 2 sec,  
25 then 72C for 3 min, followed by 20 cycles of 94C for 2 sec, and 67C for 3 min, and  
finally 4 min at 67C, and then held at 4C. Approximately 10  $\Phi$ l of each reaction  
were run on a 0.8% agarose gel, and bands (usually 500bp or larger) were excised,  
purified with the Sephaglas BandPrep kit (Pharmacia, Piscataway, NJ) and cloned  
into the TA vector pCR2.1 (Invitrogen, San Diego, California). Clones were  
30 sequenced for verification.

### Example 2: Expression Data Using Promoter Sequences

A transient expression assay was used to test the cloned DNAs for promoter activity. The promoters for PR-1#52 and PR-1#83 were recloned into a GUS expression vector (Figure 1). The ubiquitin promoter, exon 1, and intron 1 (UBI1ZM, EX1UBI1ZM, and INT1UBI1ZM) from PHP3953 were removed. The promoters were then cloned into the PstI site, 5p at bp424 in PHP3953 and the NcoI site, 3p, at bp 2430 in PHP3953. Promoter fragments were prepared for cloning into the vector by PCR with an AP1 primer to which a PstI site had been added, 5p, and the appropriate gsp2 primer, to which an NcoI site had been added 3p. The PR-1#83 promoter has been cloned in front of the CRC reporter construct, as well.

Transient data from leaf sheath explants bombarded with the PR-1#83::CRC reporter construct exhibited UV-C light inducibility, indicating promoter activity. This expression was dramatically enhanced when the PR-1#83::CRC construct was cobombarded with a ubi::avrRxv construct.

### Example 3: Expression of the Maize PR-1 Genes

In order to predict where and under what conditions the PR-1 promoters may be active, we investigated the patterns of expression of the five maize PR-1 genes with Northern blots. cDNA sequences for the PR-1 genes designated PR-1#52, PR-1#70, PR-1#81, PR-1#83, and PR-1#93 are set forth in SEQ ID NOs: 6, 8, 10, 12, and 14, respectively. Probable translation start codons for the four novel genes PR-1#52, PR-1#70, PR-1#81, and PR-1#93 are based on comparisons to published homologs, mostly from barley. PR-1#83 is homologous to a known maize PR-1 gene, the cDNA of which is published as GenBank Accession No. U82200.

Because the cDNA sequences showed significant homology, we made gene-specific probes using the following PCR primers, of which the reverse primers were designed to amplify fragments from the 5p ends of the representative cDNAs:

PR-1#52:

DO 20479 5p-gct acc aca caa aaa ctt cat att tg-3p (forward primer) (SEQ ID NO: 28)

- 5 DO 20480 5p-gca tca cgg cga cca tgg cca ac-3p (reverse primer) ((SEQ ID NO: 29)

PR-1#70:

- DO 20485 5p-agg cgg cag cgg cca aga ccg gtg t-3p (forward primer) (SEQ ID NO: 30)

DO 21053 5p-ggt gtc cct ctc gcc ctc gcc gtg g-3p (reverse primer) (SEQ ID NO: 31)

PR-1#81:

- 15 DO 20483 5p-ccc acg cgt ccg gaa gct aca atc c-3p (forward primer) (SEQ ID NO: 32)

DO 20484 5p-gcc acc ggc gac gcg atc tcg ag-3p (reverse primer) (SEQ ID NO: 33)

PR-1#83:

- 20 DO 20477 5p-cat tgg act tgc act ggt gct tgc-3p (forward primer) (SEQ ID NO: 34)  
DO 20478 5p-gcg cca cga cga tgg ctg cca tg-3p (reverse primer) (SEQ ID NO: 35)

PR-1#93:

- DO 20481 5p-gaa taa cta atc aag atc gat cg-3p (forward primer) (SEQ ID NO: 36)  
25 DO 20482 5p-cgc cga ggc ggt cgc ggc cat c-3p (reverse primer) (SEQ ID NO: 37)

RNA was isolated from maize tissues using a guanidine thiocyanate/phenol/ chloroform extraction procedure (Chomczynski and Sacchi (1987) *Anal. Biochem.* 162:156-159). Total RNA (12-30  $\Phi$ g/lane, depending upon the experiment) was electrophoresed in formaldehyde-agarose gels run according to Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold

Spring Harbor Laboratory Press, Plainview, New York). Gels were blotted using a Turboblotter (Schleicher and Schuell) overnight onto ZetaProbe (Bio-Rad). Gel-purified, gene-specific probes (above) were labeled by randomly-primed incorporation of  $^{32}\text{P}$ -ATP (see Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York) catalyzed by Klenow fragment of DNA polymerase. Unincorporated label was removed from the probes with small Sepharose CL-6B (Pharmacia) spin-columns and hybridized overnight at 65C in roller bottles (Hybaid) in 0.5M sodium phosphate, pH 7.5, 7% (w/v) SDS. Blots were washed 2x20 min at room temperature in 40mM sodium phosphate, pH 7.5, 5% SDS, and then 2x20 min at 65C in 40mM sodium phosphate, pH 7.5, 1% SDS, then either exposed to xray film (BioMax, Kodak) or phosphoimaging screen (Molecular Dynamics).

Initial tissues analyzed for expression of the PR-1 proteins included:

- X dark-grown shoots (B73, which is somewhat susceptible to earmold; about 5-days-old) inoculated with *Fusarium moniliforme* spores
- X HG11, ED42 (both very susceptible to earmold) and HT1 (resistant to earmold) silks (nonpollinated) inoculated with *F. moniliforme* spores
- X HYII culture cells treated with chitosan, a crude preparation of chitin (from crab shells) that mimics some of the effects of fungal elicitor preparations (fungal cell walls are chitinous)
- X HG11, B73, and HT1 developing kernels treated with *F. moniliforme* spores
- X leaves from families segregating lesion mimic (*Les*) mutations; these families have enhanced resistance to leaf blight caused by *Cochliobolus heterostrophus*.

These experiments reflect an interest in promoters that are regulated by fungal pathogens. Preliminary results of these initial analyses were as follows.

PR-1#52 mRNA was not detected in any of the above tissues. Library

distribution of this cDNA, as seen in a large-scale sequencing effort, indicates that this transcript is relatively rare. The material used to generate this library can be assumed to be tissue containing a variety of transposon-induced mutations.

PR-1#70 mRNA was not detected in any of the above tissues. Library  
5 distribution of this cDNA, as seen in a large-scale sequencing effort, indicates that this clone is preferentially expressed in roots, and in leaves treated with jasmonic acid (JA) or salicylic acid (SA).

PR-1#81 mRNA accumulated dramatically (approximately 50-fold greater  
than the control) in HYII culture cells 12 hours after treatment with chitosan. *F.*  
10 *moniliforme* (Fmo) inoculation and chitooligosaccharide treatment induced strong expression of PR-1#81 in the GS3 suspension cell system at 2 hours (10- to 15-fold greater compared to controls), persisting to 12 hours. Fmo inoculation induced PR-1#81 mRNA accumulation in silks, but in an inbred-dependent manner: the transcript was detectable by 1 day in ED42 silks, and was abundant by  
15 4 days; the transcript appeared at the level seen in ED42 at 4 days as early as 2 days in HT1; it did not accumulate to detectable levels in HG11 silks by 4 days after inoculation. Very little or no PR-1#81 message was seen in controls. Developing (liquid endosperm stage and later) kernels inoculated with Fmo spores also accumulated PR-1#81 mRNA in an inbred-dependent fashion: strong,  
20 constitutive (in controls and Fmo-treated samples) expression was seen at all time points (2 hours, 4 hours, 1 day, 4 days) in HG11; weaker, constitutive expression was seen at 2 hours and 4 hours in B73, but then Fmo-treated kernels accumulated steadily more PR-1#81 mRNA than controls, so that, by 4 days, inoculated kernels contained about 5- to 10-fold more transcript. PR-1#81 expression was strong and  
25 constitutive in dark-grown, B73 seedlings. Library distribution of the clones in this contig indicates that it is abundant and expressed in a variety of tissues, especially those experiencing stress (e.g., culture cells and insect-infested roots).

PR-1#83 mRNA was detectable in HT1 silks 2 days after Fmo treatment. Some transcript was detectable in ED42 silks 4 days after Fmo inoculation. This  
30 mRNA was very strongly expressed in *Les9* and *LesMT12*, lesion-containing leaves; none was seen in leaves of the same age from wild-type sibs. A low, constitutive level of PR-1#83 mRNA was seen in HG11 developing kernels.

Transcript was also detectable in both B73 and HT1 kernels 4 days after Fmo inoculation. PR-1#83 also appeared to be constitutively expressed in dark-grown, B73 seedlings, but much less so than PR-1#81. In addition, it accumulated in response to Fmo inoculations by 1 day, to levels about 5- to 10-fold over control.

5 Library distribution of the clones in

this contig indicates that it is found in leaf libraries, especially the TUSC and *Les9* libraries.

PR-1#93 mRNA was detected only in the HG11 silks treated with Fmo spores, mildly and at 2 days after inoculation. Expression in developing kernels has not yet been examined. The pattern of expression observed for PR-1#83 in dark-grown B73 shoots matched that seen for PR-1#93. Library distribution of the clones in this contig indicates that it is also root-preferred and seen in log-phase BMS cells.

15 PR-1 mRNA accumulation was examined further in the following tissues:

- X JA- (jasmonic acid) or SA- (salicylic acid) treated leaves
- X UV-treated leaves
- X a variety of tissues from a couple of standard inbreds (HH60 and A63; e.g., roots, husks, silks, leaves, ears, tassels, etc.)
- 20 X *F. moniliforme*-inoculated germinating embryos

Results were as follows.

PR-1#52 expression was not induced in leaves by JA or SA treatments. Expression was induced very weakly by UV light, as early as 24 hours after treatment, and persisted until at least 4 days after irradiation. New Northern blots show PR-1#52 was mildly expressed in seedling roots, mature leaf blades, husks, and *Les9* leaf tissues containing lesions.

PR-1#70 expression was not induced in leaves by JA, SA, or UV-light treatments. New Northern blots show very weak expression of PR-1#70 in immature ears and husks and strong expression in cobs from maturing ears (about 23 days after pollination (dap)) and in roots from month-old plants.

PR-1#81 expression was not induced in leaves by JA and SA treatments, or immediately following UV-light treatments. Expression was mildly induced by 3

hours after UV-light treatment, about 3-fold more than that seen for PR-1#52 by 24 hours. This UV-induced expression was somewhat reduced by 4 days. PR-1#81 was constitutively expressed in kernels at 23 dap (i.e., expression wasn't induced by *Fusaria moniliforme* (Fmo) inoculation). PR-1#81 was much more strongly expressed in this tissue in HG11 kernels versus HT1 kernels. Expression was dramatically induced above a substantial background by Fmo treatment in mature HH60 embryos, which had been excised from seeds imbibed overnight and allowed to germinate, as early as 2 hours. New Northern blots show that PR-1#81 is mildly expressed in seedling roots, mature leaves, husks, 23 and 40 dap kernels, and cobs from maturing ears; it is very strongly expressed in roots of month-old plants.

PR-1#83 expression was mildly induced in A63 leaves 8 hours after infiltration with SA, and mildly induced within 6 hours of a topical spray with JA. Expression was very strongly induced in leaves by UV light treatment: accumulation began by 3 hours and increased about 20- to 30-fold by 1 day. In interactions with *Cochliobolus carbonum*; expression was significantly induced by 4 days into a susceptible interaction (HC-toxin-producing *C. carbonum* on Pr leaves), but only very mildly induced by 4 days in an incompatible interaction (toxin- *C. carbonum* on Pr leaves). Expression was not induced in the excised embryo system. New Northern blots show that PR-1#83 was mildly expressed in roots of seedlings and month-old plants and husks; it was very strongly expressed in mature leaf blades.

PR-1#93 was constitutively expressed in green leaves, regardless of UV light, SA, or JA treatments. Its expression was seen in all tissues surveyed, including immature ears, week-old seedling leaves, seedling roots, mature blades, husks, 23 and 40 dap kernels, and silks. Expression was fairly abundant in roots from month-old plants and in cob.

Except for PR-1 accumulation in lesion mimic leaves, these observations are novel. San Segundo's reports concerning PRs show that it is induced by Fmo, but its expression is restricted to the embryos and the aleurone of germinating maize seeds (Casacuberta *et al.* (1992) *Mol. Gen. Genet.* 234:97-104).

**Example 4: Transformation and Regeneration of Transgenic Plants**

A maize PR-1 gene of the invention is cloned into a plant expression vector as shown in Figure 2. The nucleotide sequence is under transcriptional control of a PR-1 inducible promoter. The selectable marker gene PAT is used.

Immature maize embryos from greenhouse donor plants are bombarded with the plasmid containing the PR-1 gene operably linked to a PR-1 inducible promoter plus a plasmid containing the selectable marker gene PAT (Wohlleben *et al.* (1988) *Gene* 70:25-37) that confers resistance to the herbicide Bialaphos. Transformation is performed as follows. All media recipes are in the Appendix.

**Preparation of Target Tissue**

The ears are surface sterilized in 30% Chlorox bleach plus 0.5% Micro detergent for 20 minutes, and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5-cm target zone in preparation for bombardment.

**Preparation of DNA**

A plasmid vector comprising the PR-1 gene operably linked to a PR-1 inducible promoter is made. This plasmid DNA plus plasmid DNA containing a PAT selectable marker is precipitated onto 1.1  $\mu\text{m}$  (average diameter) tungsten pellets using a  $\text{CaCl}_2$  precipitation procedure as follows:

- 100  $\mu\text{l}$  prepared tungsten particles in water
- 10  $\mu\text{l}$  (1  $\mu\text{g}$ ) DNA in TrisEDTA buffer (1  $\mu\text{g}$  total)
- 100  $\mu\text{l}$  2.5 M  $\text{CaCl}_2$
- 10  $\mu\text{l}$  0.1 M spermidine

Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol, and centrifuged for 30 seconds. Again the liquid is removed, and 105  $\mu$ l 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the tungsten/DNA particles are briefly sonicated and 10  $\mu$ l spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment.

10

#### Particle Gun Treatment

The sample plates are bombarded at level #4 in particle gun #HE34-1 or #HE34-2. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA.

15

#### Subsequent Treatment

Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos, and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed somatic embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5" pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity. Plants are monitored and scored for enhanced disease resistance.

20

25

WO 99/43819

PCT/US99/03011

45

**APPENDIX****272 V**

| Ingredient                    | Amount  | Unit |
|-------------------------------|---------|------|
| D-I H <sub>2</sub> O          | 950.000 | ml   |
| MS Salts (GIBCO 11117-074)    | 4.300   | g    |
| Myo-Inositol                  | 0.100   | g    |
| MS Vitamins Stock Solution ## | 5.000   | ml   |
| Sucrose                       | 40.000  | g    |
| Bacto-Agar @                  | 6.000   | g    |

**Directions:**

- 5 @ = Add after bringing up to volume

Dissolve ingredients in polished D-I H<sub>2</sub>O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H<sub>2</sub>O after adjusting pH

Sterilize and cool to 60°C.

- 10 ## = Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H<sub>2</sub>O in sequence. Bring up to volume with polished D-I H<sub>2</sub>O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.
- 15 Total Volume (L) = 1.00

WO 99/43819

PCT/US99/03011

46

288 J

| Ingredient                    | Amount  | Unit |
|-------------------------------|---------|------|
| D-I H <sub>2</sub> O          | 950.000 | ml   |
| MS Salts                      | 4.300   | g    |
| Myo-Inositol                  | 0.100   | g    |
| MS Vitamins Stock Solution ## | 5.000   | ml   |
| Zeatin .5mg/ml                | 1.000   | ml   |
| Sucrose                       | 60.000  | g    |
| Gelrite @                     | 3.000   | g    |
| Indoleacetic Acid 0.5 mg/ml # | 2.000   | ml   |
| .1mM Absciscic Acid           | 1.000   | ml   |
| Bialaphos 1mg/ml #            | 3.000   | ml   |

**Directions:****@ = Add after bringing up to volume**

- 5 Dissolve ingredients in polished D-I H<sub>2</sub>O in sequence

Adjust to pH 5.6

Bring up to volume with polished D-I H<sub>2</sub>O after adjusting pH

Sterilize and cool to 60°C.

Add 3.5g/L of Gelrite for cell biology.

- 10 ## = Dissolve 0.100 g of Nicotinic Acid; 0.020 g of Thiamine.HCL; 0.100 g of Pyridoxine.HCL; and 0.400 g of Glycine in 875.00 ml of polished D-I H<sub>2</sub>O in sequence. Bring up to volume with polished D-I H<sub>2</sub>O. Make in 400 ml portions. Thiamine.HCL & Pyridoxine.HCL are in Dark Desiccator. Store for one month, unless contamination or precipitation occurs, then make fresh stock.
- 15 Total Volume (L) = 1.00

WO 99/43819

PCT/US99/03011

47

**560 R**

| Ingredient                               | Amount  | Unit |
|------------------------------------------|---------|------|
| D-I Water, Filtered                      | 950.000 | ml   |
| CHU (N6) Basal Salts (SIGMA C-1416)      | 4.000   | g    |
| Eriksson's Vitamin Mix (1000X SIGMA-1511 | 1.000   | ml   |
| Thiamine.HCL 0.4mg/ml                    | 1.250   | ml   |
| Sucrose                                  | 30.000  | g    |
| 2, 4-D 0.5mg/ml                          | 4.000   | ml   |
| Gelrite @                                | 3.000   | g    |
| Silver Nitrate 2mg/ml #                  | 0.425   | ml   |
| Bialaphos 1mg/ml #                       | 3.000   | ml   |

**Directions:****@ = Add after bringing up to volume****5 # = Add after sterilizing and cooling to temp.****Dissolve ingredients in D-I H<sub>2</sub>O in sequence****Adjust to pH 5.8 with KOH****Bring up to volume with D-I H<sub>2</sub>O****Sterilize and cool to room temp.****10 Total Volume (L) = 1.00**

WO 99/43819

PCT/US99/03011

48

560 Y

| Ingredient                               | Amount  | Unit |
|------------------------------------------|---------|------|
| D-I Water, Filtered                      | 950.000 | ml   |
| CHU (N6) Basal Salts (SIGMA C-1416)      | 4.000   | g    |
| Eriksson's Vitamin Mix (1000X SIGMA-1511 | 1.000   | ml   |
| Thiamine.HCL 0.4mg/ml                    | 1.250   | ml   |
| Sucrose                                  | 120.000 | g    |
| 2,4-D 0.5mg/ml                           | 2.000   | ml   |
| L-Proline                                | 2.880   | g    |
| Gelrite @                                | 2.000   | g    |
| Silver Nitrate 2mg/ml #                  | 4.250   | ml   |

**Directions:****@ = Add after bringing up to volume****5 # = Add after sterilizing and cooling to temp.****Dissolve ingredients in D-I H<sub>2</sub>O in sequence****Adjust to pH 5.8 with KOH****Bring up to volume with D-I H<sub>2</sub>O****Sterilize and cool to room temp.****10 \*\* Autoclave less time because of increased sucrose\*\*****Total Volume (L) = 1.00**

15 All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

5

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WO 99/43819

PCT/US99/03011

## SEQUENCE LISTING

&lt;110&gt; Crane, Virginia

&lt;120&gt; Family Of Maize PR-1 Genes And Promoters

&lt;130&gt; 5718-32, 035718/175219

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 37

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

&lt;211&gt; 881

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;400&gt; 1

cgacggcccg ggctggtatc catgtcaatt ttgctgctac cgtcgggcga tcgcgtcctt 60

ggaattcttg cccctgtgga atttcttcta gctactaata acttgccgtc actgttgatt 120

gaccaaattg gcagctactt tttgcagttt aattatactc tgtttcccc gatttgctac 180

ttactttgat cacaaatata tatttattta catttgttct aagtcagttt ttgataagct 240

aagaatatga aagtttttat tgtgcgcaaa cataagactt cccattatta gcttcacgt 300

aaaaaataac atataagtac tcttttttta atatagcata aattttatat cggatactc 360

ttttattcaa tataatatag tataataatg acacatgtat aagtggccta ctattacaga 420

ctatatcgat cttctaaaag actttttaatt tgaaacctag ttggttaaatt ttttcagagc 480

tgcttttgct taaataaact aaggatctat tttagtttgt gactaactat gccacatttt 540

gcctaagggtt agtcattcca attgaagaac tatcttttagg cacaaaagtg taacaaaatg 600

tgacaaatta gcaggcgaac caaacagacc ctaaaagcaa ccaaagagt tgttttctac 660

ccaagtactt caatggaagc agcttcttct ctagagcatc cttagatctg cttctctaga 720

gcatcctttg acatatcgat tgacaagcta gtcgccaagg tagccttttg ctaccactat 780

aaaaatacct gggcacacac gccacaactc acgcatccat ctaccacaca agaaacttca 840

WO 99/43819

PCT/US99/03011

tatttggttca ttgtctcca gcaatacggg ttccaacccat g

881

&lt;210&gt; 2

&lt;211&gt; 1199

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;400&gt; 2

cgacggcccg ggctggttca aaaaatagag agagttagct cttatgcaat tgacaagaaa 60

tttatataag agcaaacttt aaagtcagct ttattatatg aatgtattta ttattattct 120

ataaatgaca tggtaaagag ttagaaccaa caataaactc tattattaaa cttgctctaa 180

acctagaagc aaagntagtt gcagattcgc agttactaaa aaaaatgtgt catttacctt 240

tgtttatcgc agctcatgcc gtcactgatg accgcatcac acacatgcct ctccatccag 300

acctgataga gtgtggtgcg taggcggcgt agctatcgta ttcgtagctc ctcggtgccg 360

cacgcttcga gaatgccact gaagtacggg gtcctgtagt actagtatat cttttggcag 420

catagacgac cgtcaccggt atttgatata gtgatggcga tttttttaac accagatgac 480

actctacaaa gattttattg aatattcaat aaaaaatact cgacaaaaaa aattatcagt 540

atacagttta tcaagacatc ttttgccgaa attcgtactt taccaaaagt tttttagact 600

ttgccaagca gttgtatcta gtagtgatag taagacttac tttcaaaca agaaaatatg 660

aaagattttt aaatggttat ttttctactt aaaaattttc tacatgaatt tttggaacac 720

aggattcaat catatcaatt ccatcgaaat tttctatgga ctgcatataa gttttggagg 780

aaaaacacaa gaaatccaac ctcttggaac cggtacgtac tatgactctt gtttctctat 840

tcaaaatcct acatagctag catccaaagg ctaggtaaga aatttccgtg cgttctcaat 900

tcatgtaggt tacaatttat agccaattct aattatatgt ttgttttatt gttctgtttt 960

tatattcctg tgttccaaag agggcctata atagaaacag gacctggaca tcgccatcgc 1020

catctgatga tctcgcaaat aataatgtag ggtatacaag agaggcagat ttgacagccg 1080

tcctactcca ttgctccta gtcccaaata ctagcaggcg gcaggcggcc aagaccggtg 1140

WO 99/43819

PCT/US99/03011

tccctcgacg acctgccgag tgccgaggca agacgcgagg aggctgtaat ggcggggcgc 1199

<210> 3  
 <211> 955  
 <212> DNA  
 <213> Zea mays

<400> 3  
 cgacggcccc ggctggtagt cttgggtggg tcagatgtgg ccgataaaga tgaaggcaag 60  
 aacaatgtca ttggcgatcc tcgcacgccca agtcagttac aaggagtggg tacttgaaag 120  
 gctctgaaca agagaatgac taataagact caaggcccta gagggcaaac acgaccggat 180  
 acccgattac gatcacatgt cttgtgtacg caggacgggc cgggacctaa ggccgatcag 240  
 tctaagagtg accaaaagca acaacgacct cagacctta gaccatgaca tctagaagaa 300  
 ggtatatgca agcaaaatac atctaaagca tctgactgac tcgttagtgc tagcccttct 360  
 tttgaacaac ttctttctaa gtatatgaat aagaaggctc tttcacacaa ttgatcgaca 420  
 aaacgatcaa tatcatccac aacgaggaag caatccatgc aaggggcaaaa gccgaataaa 480  
 tcggcccagg aagtgggtgca accaatgtcg cctactcatc cgctctagga atgtcgtgtt 540  
 actttccacc agtctactca tcgatgatgt tttatcctgc tgacatgtga aaaagtatga 600  
 cgatgaatcc gtatcacaca ggggcggacg cagagggagg caaagtgggt catagccacc 660  
 tcaattttta tgatatttta tatatcatga cgtgcagtct ctttgcaacc ccagccacat 720  
 taattaatag actccaccga cgagcgacga gtgatggtac cggccgcccgg cccaggccaa 780  
 cccaagtgga aaaggccgac gactcccgga cgtctcatcc tcaccggacg ccaccaaccc 840  
 ccgcaatctc cagacgtacg agccgcctat tttaaagccct cagtctgcca ctctcatggc 900  
 aacgcaagca gaagctacaa tcctaaaacc atctgcttca gccttcagct agccc 955

<210> 4  
 <211> 1149  
 <212> DNA  
 <213> Zea mays

<400> 4

WO 99/43819

PCT/US99/03011

agtcgacggc ccgggctggt atctaagtat aatcgtatTT ataaataatt tccgaggccc 60  
 cttttcctgt ctgatgaaga tcgctgaggc tgaacatgaa atgacagcag gtttctttat 120  
 tggccaagtc ccttgagaaa acctaattgt aaacaattag tcttctagaa ccctagcgaa 180  
 ccttgtagcgc gttaagaaac ggattgggtca agccagccga gatcatatga agtagatctt 240  
 ccgatagtaa ggaggtgctt ggtttgagga atcatttcat ccaaaatgta gtgatgcac 300  
 atgggtccat tcctcaaatt tgggtgggatg acctcatccc tcatattagt actaactaaa 360  
 taactataag gaatgaggtg atgatggatc aactcaatcc attccacaaa ccaaacaaaa 420  
 aagtgaggag tgagaagacg atggactaga tcattcctca aaccaaacag ctcataaagt 480  
 tcaaaactag agttgagaat aaaaactata gtagcatga gcattaatct cgggtctcaag 540  
 ttttatctgg ataaagagtc catattagtc ctggttgtgg ttattcgaca aaatgtggat 600  
 cctttatcaa ttggaactaa ttaagggatt ttcacatgcc agttccgaag aaaagcggtc 660  
 tattcaccta ctatgtaggt gaggtaaaaa aaactactct gctaataata cttcattcct 720  
 tcttttttat ttttcatatt ttaatttgaa aataaattaa ggatgaccaa tatttaagaa 780  
 tagatgtagt atatgagatg acgggttcaa gccttccaag ccaattttcc tgcttgtgtt 840  
 ctctagaatg ccgtgcccgt ccattttgta cctcctgctc ccagagtcta gtaaacgaaa 900  
 tccatgttta tccgaaaact ttctgcca tcctatactg ttactcgcta atttattttt 960  
 aaactaaacc acgataaata aaaaaaacgg atgaagtata tgctatccaa taataccgtg 1020  
 gaaattcttg gctcccttg aaggtcttga aggcgccacc attgaccgtg accatgccaa 1080  
 gtgcctacct tctgcaaatt atgctatata tcttgcttgg acttgcccct atatacgatt 1140  
 ataaatacc 1149

&lt;210&gt; 5

&lt;211&gt; 255

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;400&gt; 5

cattctcgat cgatcttgat tagttattct tatatgagat gcatgggaga gagctaggtc 60

WO 99/43819

PCT/US99/03011

gtgttccggg tttttatagg cagtctctct ctctacatat ataatgggta ggtagctgag 120  
 tgagcgcagt gtgcgacttt gcgtgttatt ttttacacac aatgtgcgaa gcatgcatta 180  
 attaactcgg cacgcaagta ctatctctat catgcatgca ccagcccggg ccgtcgacca 240  
 cgcgtgccct atagt 255

&lt;210&gt; 6

&lt;211&gt; 866

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (111)..(581)

&lt;400&gt; 6

cccacgcgtc cgctaccaca caaaaacttc atatttggtc atttgtctcc agcaatataa 60

tggattactc gtctactagg gtcgtcgtgt gtttagctct ggcgttggcc atg gtc 116  
 Met Val  
 1

gcc gtg atg ccg tgc gcg gcc cag aac tcg ccg cag gac ttc gtg aac 164  
 Ala Val Met Pro Cys Ala Ala Gln Asn Ser Pro Gln Asp Phe Val Asn  
 5 10 15

ccg cac aat gcg gcg cgc gcg gcc gtg ggc gtc ggc ccg gtg tcg tgg 212  
 Pro His Asn Ala Ala Arg Ala Ala Val Gly Val Gly Pro Val Ser Trp  
 20 25 30

gat gag aac gtg gcc gcc ttc gcg cgg agc tac gcc gcg cag cgc cag 260  
 Asp Glu Asn Val Ala Ala Phe Ala Arg Ser Tyr Ala Ala Gln Arg Gln  
 35 40 45 50

ggc gac tgc aag ctg gtg cac tcc ggc ggc ggg ccc aac cac tac ggg 308  
 Gly Asp Cys Lys Leu Val His Ser Gly Gly Gly Pro Asn His Tyr Gly  
 55 60 65

gag aac atc ttc tgg ggc ggc ggc agc gcc tgg aag gcg tcg gac gcc 356  
 Glu Asn Ile Phe Trp Gly Gly Gly Ser Ala Trp Lys Ala Ser Asp Ala  
 70 75 80

gtc ggc ttg tgg gtg ggg gag aag cag aac tac gat tac aac agc aac 404  
 Val Gly Leu Trp Val Gly Glu Lys Gln Asn Tyr Asp Tyr Asn Ser Asn

WO 99/43819

PCT/US99/03011

| 85                                                                  | 90  | 95  |     |
|---------------------------------------------------------------------|-----|-----|-----|
| agc tgc gcg gcg ggg aag gtg tgc ggc cac tac acg caa gtc gtc tgg     |     |     | 452 |
| Ser Cys Ala Ala Gly Lys Val Cys Gly His Tyr Thr Gln Val Val Trp     |     |     |     |
| 100                                                                 | 105 | 110 |     |
| cgc aaa tcc ccg cca tcg gct gcg ccc gcg tcg tct gca aca acg gcg     |     |     | 500 |
| Arg Lys Ser Pro Pro Ser Ala Ala Pro Ala Ser Ser Ala Thr Thr Ala     |     |     |     |
| 115                                                                 | 120 | 125 | 130 |
| gtg gcg tct tca tca cct gca act aca acc cgc cgg gca act tcc gcg     |     |     | 548 |
| Val Ala Ser Ser Ser Pro Ala Thr Thr Thr Arg Arg Ala Thr Ser Ala     |     |     |     |
|                                                                     | 135 | 140 | 145 |
| gac aga gac cct act agc tac cta gct agc tag ttccaatatc gattatacgc   |     |     | 601 |
| Asp Arg Asp Pro Thr Ser Tyr Leu Ala Ser                             |     |     |     |
|                                                                     | 150 | 155 |     |
| gtacatatatc tctacatgca tgttcagatc gatgatatat atacctcgcg tgcattgctac |     |     | 661 |
| cgattgaata aaccacaaac taagcttagg ccaccagttg attaggttta aagtgaacct   |     |     | 721 |
| actcaccag gtttaagtga agtgtgcata catgtatgtg tatgttctgt gtgggggtgtg   |     |     | 781 |
| acagtttgtt tgggtgtttca taaaataaat aaatgaatga ataataagag tgttcattgag |     |     | 841 |
| caaaaaaaaa aaaaaaaaaa aaaaa                                         |     |     | 866 |

&lt;210&gt; 7

&lt;211&gt; 156

&lt;212&gt; PRT

&lt;213&gt; Zea mays

&lt;400&gt; 7

Met Val Ala Val Met Pro Cys Ala Ala Gln Asn Ser Pro Gln Asp Phe

1

5

10

15

Val Asn Pro His Asn Ala Ala Arg Ala Ala Val Gly Val Gly Pro Val

20

25

30

Ser Trp Asp Glu Asn Val Ala Ala Phe Ala Arg Ser Tyr Ala Ala Gln

35

40

45

Arg Gln Gly Asp Cys Lys Leu Val His Ser Gly Gly Gly Pro Asn His

50

55

60

Tyr Gly Glu Asn Ile Phe Trp Gly Gly Gly Ser Ala Trp Lys Ala Ser

WO 99/43819

PCT/US99/03011

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 65  |     | 70  |     | 75  |     | 80  |     |     |     |     |     |     |     |     |     |
| Asp | Ala | Val | Gly | Leu | Trp | Val | Gly | Glu | Lys | Gln | Asn | Tyr | Asp | Tyr | Asn |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ser | Asn | Ser | Cys | Ala | Ala | Gly | Lys | Val | Cys | Gly | His | Tyr | Thr | Gln | Val |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Val | Trp | Arg | Lys | Ser | Pro | Pro | Ser | Ala | Ala | Pro | Ala | Ser | Ser | Ala | Thr |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Thr | Ala | Val | Ala | Ser | Ser | Ser | Pro | Ala | Thr | Thr | Thr | Arg | Arg | Ala | Thr |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Ser | Ala | Asp | Arg | Asp | Pro | Thr | Ser | Tyr | Leu | Ala | Ser |     |     |     |     |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     |     |

&lt;210&gt; 8

&lt;211&gt; 973

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (102)..(797)

&lt;400&gt; 8

tcgaccacgc gtccggtccc aatactagca ggcggcagcg gccaaagaccg gtgtccctcg 60

acgacctgcc gagtgccgag gcaagacgcg aggaggctgt a atg gcg ggc gcc gag 116

Met Ala Gly Ala Glu

1

5

gtg gga gaa gac aag tac cgc tcc ttc atc cac ggc gag ggc gag agg 164

Val Gly Glu Asp Lys Tyr Arg Ser Phe Ile His Gly Glu Gly Glu Arg

10

15

20

gac acc gtg tgg agg tac ggc gcc ccg ccc aac tac gac gtg gtc aac 212

Asp Thr Val Trp Arg Tyr Gly Ala Pro Pro Asn Tyr Asp Val Val Asn

25

30

35

aag ctc ttc gag gaa gag agg act cag gtg tgg ccc gag ggc tcg ctg 260

Lys Leu Phe Glu Glu Glu Arg Thr Gln Val Trp Pro Glu Gly Ser Leu

40

45

50

gag gag aag gtg cag cgg ctg ctc aag agc tgg gag atg gag ttg gtg 308

Glu Glu Lys Val Gln Arg Leu Leu Lys Ser Trp Glu Met Glu Leu Val

WO 99/43819

PCT/US99/03011

|                                                                   |     |     |     |
|-------------------------------------------------------------------|-----|-----|-----|
| 55                                                                | 60  | 65  |     |
| cac aag gcg cgg ccc gag gac cag aag acc gtc aac tcg gag aaa tac   |     |     | 356 |
| His Lys Ala Arg Pro Glu Asp Gln Lys Thr Val Asn Ser Glu Lys Tyr   |     |     |     |
| 70                                                                | 75  | 80  | 85  |
| tct gcc agc acc aac ggg atg agc gct ctg acc cgg gcc gag gtg atg   |     |     | 404 |
| Ser Ala Ser Thr Asn Gly Met Ser Ala Leu Thr Arg Ala Glu Val Met   |     |     |     |
|                                                                   | 90  | 95  | 100 |
| gcc atc ggc ggc tac aac aac ttc ctg cgc acc aag ctg ccc ccg gag   |     |     | 452 |
| Ala Ile Gly Gly Tyr Asn Asn Phe Leu Arg Thr Lys Leu Pro Pro Glu   |     |     |     |
|                                                                   | 105 | 110 | 115 |
| cac cgc atc tac gac ccg gac agc gag acc gtg gag tcc gcc atg gcc   |     |     | 500 |
| His Arg Ile Tyr Asp Pro Asp Ser Glu Thr Val Glu Ser Ala Met Ala   |     |     |     |
|                                                                   | 120 | 125 | 130 |
| acc ttc acc acg gcc ttc ccg cgg ggc ttc gcc atc gag gtg ctc gac   |     |     | 548 |
| Thr Phe Thr Thr Ala Phe Pro Arg Gly Phe Ala Ile Glu Val Leu Asp   |     |     |     |
|                                                                   | 135 | 140 | 145 |
| gtc tac agc ggc ccg ccc agg atc gcc ttc aag ttc cgc cac tgg ggc   |     |     | 596 |
| Val Tyr Ser Gly Pro Pro Arg Ile Ala Phe Lys Phe Arg His Trp Gly   |     |     |     |
| 150                                                               | 155 | 160 | 165 |
| tac atg gag ggg ccc ttc aag ggc cac ccg ccg cac ggc cag cgg gtc   |     |     | 644 |
| Tyr Met Glu Gly Pro Phe Lys Gly His Pro Pro His Gly Gln Arg Val   |     |     |     |
|                                                                   | 170 | 175 | 180 |
| gag ctc ttc ggc gtc tgc atc ttc cat gtt gac gaa gac atg aag gtg   |     |     | 692 |
| Glu Leu Phe Gly Val Cys Ile Phe His Val Asp Glu Asp Met Lys Val   |     |     |     |
|                                                                   | 185 | 190 | 195 |
| gac aag tca gag tac ttc tac gag cgc ggc aac ttc ctc gcc ggc ttc   |     |     | 740 |
| Asp Lys Ser Glu Tyr Phe Tyr Glu Arg Gly Asn Phe Leu Ala Gly Phe   |     |     |     |
|                                                                   | 200 | 205 | 210 |
| ttg agt gcc cct gcc cct gat ggc tca ggc ggt tgc ccc gtg atg cgc   |     |     | 788 |
| Leu Ser Ala Pro Ala Pro Asp Gly Ser Gly Gly Cys Pro Val Met Arg   |     |     |     |
|                                                                   | 215 | 220 | 225 |
| ggg aac tga atgggcctgc aatgggacat tggaagacta ggcattggaa           |     |     | 837 |
| Gly Asn                                                           |     |     |     |
| 230                                                               |     |     |     |
| cattgggatt attagcatta gcgatcccga atccgctttt attacggttt aataattcca |     |     | 897 |

WO 99/43819

PCT/US99/03011

tcatgtaaca tgacacttgc atttgtgttt gaataaacat ttcagttgct tccaaaaaaaa 957

aaaaaaaaaa aaaaaa

973

&lt;210&gt; 9

&lt;211&gt; 231

&lt;212&gt; PRT

&lt;213&gt; Zea mays

&lt;400&gt; 9

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Gly | Ala | Glu | Val | Gly | Glu | Asp | Lys | Tyr | Arg | Ser | Phe | Ile | His |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Glu | Gly | Glu | Arg | Asp | Thr | Val | Trp | Arg | Tyr | Gly | Ala | Pro | Pro | Asn |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Asp | Val | Val | Asn | Lys | Leu | Phe | Glu | Glu | Glu | Arg | Thr | Gln | Val | Trp |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Glu | Gly | Ser | Leu | Glu | Glu | Lys | Val | Gln | Arg | Leu | Leu | Lys | Ser | Trp |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Met | Glu | Leu | Val | His | Lys | Ala | Arg | Pro | Glu | Asp | Gln | Lys | Thr | Val |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Ser | Glu | Lys | Tyr | Ser | Ala | Ser | Thr | Asn | Gly | Met | Ser | Ala | Leu | Thr |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ala | Glu | Val | Met | Ala | Ile | Gly | Gly | Tyr | Asn | Asn | Phe | Leu | Arg | Thr |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Pro | Pro | Glu | His | Arg | Ile | Tyr | Asp | Pro | Asp | Ser | Glu | Thr | Val |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Ala | Met | Ala | Thr | Phe | Thr | Thr | Ala | Phe | Pro | Arg | Gly | Phe | Ala |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Glu | Val | Leu | Asp | Val | Tyr | Ser | Gly | Pro | Pro | Arg | Ile | Ala | Phe | Lys |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Arg | His | Trp | Gly | Tyr | Met | Glu | Gly | Pro | Phe | Lys | Gly | His | Pro | Pro |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Gly | Gln | Arg | Val | Glu | Leu | Phe | Gly | Val | Cys | Ile | Phe | His | Val | Asp |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     |     | 190 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Asp | Met | Lys | Val | Asp | Lys | Ser | Glu | Tyr | Phe | Tyr | Glu | Arg | Gly | Asn |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

WO 99/43819

PCT/US99/03011

|                                                                      |     |     |  |
|----------------------------------------------------------------------|-----|-----|--|
| 195                                                                  | 200 | 205 |  |
| Phe Leu Ala Gly Phe Leu Ser Ala Pro Ala Pro Asp Gly Ser Gly Gly      |     |     |  |
| 210                                                                  | 215 | 220 |  |
| Cys Pro Val Met Arg Gly Asn                                          |     |     |  |
| 225                                                                  | 230 |     |  |
| <br>                                                                 |     |     |  |
| <210> 10                                                             |     |     |  |
| <211> 887                                                            |     |     |  |
| <212> DNA                                                            |     |     |  |
| <213> Zea mays                                                       |     |     |  |
| <br>                                                                 |     |     |  |
| <220>                                                                |     |     |  |
| <221> CDS                                                            |     |     |  |
| <222> (93)..(575)                                                    |     |     |  |
| <br>                                                                 |     |     |  |
| <400> 10                                                             |     |     |  |
| cccacgcgtc cggaagctac aatcctaaaa ccattctgctt cagccttcag ctagcccca 60 |     |     |  |
| <br>                                                                 |     |     |  |
| gttttagtcgg ccgatcgatc actgaagtag ta atg gcc tcc gcc aac agc tgg 113 |     |     |  |
| Met Ala Ser Ala Asn Ser Trp                                          |     |     |  |
| 1 5                                                                  |     |     |  |
| <br>                                                                 |     |     |  |
| acc ctc gag atc gcg tcg ccg gtg gct ccg cag cgc ctg ttc cgc gcc 161  |     |     |  |
| Thr Leu Glu Ile Ala Ser Pro Val Ala Pro Gln Arg Leu Phe Arg Ala      |     |     |  |
| 10 15 20                                                             |     |     |  |
| <br>                                                                 |     |     |  |
| gcc gtg atg gac tgg cac acc ctg gcg ccc aag gtc gcc tcc cac gtc 209  |     |     |  |
| Ala Val Met Asp Trp His Thr Leu Ala Pro Lys Val Ala Ser His Val      |     |     |  |
| 25 30 35                                                             |     |     |  |
| <br>                                                                 |     |     |  |
| gtc gcc agc gcg cag ccc gtg gag ggc gac ggc ggc gtt ggc agc gtc 257  |     |     |  |
| Val Ala Ser Ala Gln Pro Val Glu Gly Asp Gly Gly Val Gly Ser Val      |     |     |  |
| 40 45 50 55                                                          |     |     |  |
| <br>                                                                 |     |     |  |
| agg cag ttc aac ttc acc tca gtc atg ccg ttc agc ttc atg aag gag 305  |     |     |  |
| Arg Gln Phe Asn Phe Thr Ser Val Met Pro Phe Ser Phe Met Lys Glu      |     |     |  |
| 60 65 70                                                             |     |     |  |
| <br>                                                                 |     |     |  |
| agg ctc gag ttc ctg gac gcg gac aag tgc gag tgc aag aac acg ctc 353  |     |     |  |
| Arg Leu Glu Phe Leu Asp Ala Asp Lys Cys Glu Cys Lys Asn Thr Leu      |     |     |  |
| 75 80 85                                                             |     |     |  |
| <br>                                                                 |     |     |  |
| atc gag ggc ggc ggc atc ggc gtc gcc atc gaa acg gcg acg tcg cac 401  |     |     |  |
| Ile Glu Gly Gly Gly Ile Gly Val Ala Ile Glu Thr Ala Thr Ser His      |     |     |  |
| 90 95 100                                                            |     |     |  |

WO 99/43819

PCT/US99/03011

atc aag gtg gag ccc gcg gcc ggc ggc ggg agc gtg gtg aag gtc gaa 449  
 Ile Lys Val Glu Pro Ala Ala Gly Gly Gly Ser Val Val Lys Val Glu  
 105 110 115

tcc act tac aag ctg ctg ccg ggc gtg gag gtg aag gac gag atc gcc 497  
 Ser Thr Tyr Lys Leu Leu Pro Gly Val Glu Val Lys Asp Glu Ile Ala  
 120 125 130 135

aag gcc aag gag tcc gtc acc gcc atc ttc aag ggt gcc gag gcc tac 545  
 Lys Ala Lys Glu Ser Val Thr Ala Ile Phe Lys Gly Ala Glu Ala Tyr  
 140 145 150

ctc gtc gcc aac ccc gac gcc tac aac taa accattggac tgggatgagc 595  
 Leu Val Ala Asn Pro Asp Ala Tyr Asn  
 155 160

ttcatttccc ttccgtcttg tttggatata caagggtatt gtgtacggag attgggtttt 655  
 ctttcccctt cttttttggt ttcatacaaa ataaaataaa gctgctctaa agcagcgtgc 715  
 ggtttttgga ctcgagatgg tatgattata tatatgtacg tgctgtcctt gtgttggtata 775  
 ccgtcatggt tttctgtgtg ggaagaaaaa gggcattctg ttttagaatc ctatgtaggt 835  
 ttgactgaat aaggaatttt acttgctcaa aaaaaaaaaa aaaaaaaaaa aa 887

<210> 11  
 <211> 160  
 <212> PRT  
 <213> Zea mays

<400> 11  
 Met Ala Ser Ala Asn Ser Trp Thr Leu Glu Ile Ala Ser Pro Val Ala  
 1 5 10 15  
 Pro Gln Arg Leu Phe Arg Ala Ala Val Met Asp Trp His Thr Leu Ala  
 20 25 30  
 Pro Lys Val Ala Ser His Val Val Ala Ser Ala Gln Pro Val Glu Gly  
 35 40 45  
 Asp Gly Gly Val Gly Ser Val Arg Gln Phe Asn Phe Thr Ser Val Met  
 50 55 60  
 Pro Phe Ser Phe Met Lys Glu Arg Leu Glu Phe Leu Asp Ala Asp Lys  
 65 70 75 80

WO 99/43819

PCT/US99/03011

Cys Glu Cys Lys Asn Thr Leu Ile Glu Gly Gly Gly Ile Gly Val Ala  
85 90 95

Ile Glu Thr Ala Thr Ser His Ile Lys Val Glu Pro Ala Ala Gly Gly  
100 105 110

Gly Ser Val Val Lys Val Glu Ser Thr Tyr Lys Leu Leu Pro Gly Val  
115 120 125

Glu Val Lys Asp Glu Ile Ala Lys Ala Lys Glu Ser Val Thr Ala Ile  
130 135 140

Phe Lys Gly Ala Glu Ala Tyr Leu Val Ala Asn Pro Asp Ala Tyr Asn  
145 150 155 160

&lt;210&gt; 12

&lt;211&gt; 749

&lt;212&gt; DNA

&lt;213&gt; Zea mays

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (92)..(583)

&lt;400&gt; 12

cccacgcgtc cgcccacgcg tccgccgatc acacattgga cttgcactgg tgcttgctca 60

taattactag ttcattcagca aacaacaaac a atg gca ccg agg cta gcg tgc 112  
Met Ala Pro Arg Leu Ala Cys  
1 5

ctc cta gct ctg gcc atg gca gcc atc gtc gtg gcg ccg tgc acg gcc 160  
Leu Leu Ala Leu Ala Met Ala Ala Ile Val Val Ala Pro Cys Thr Ala  
10 15 20

cag aac tcg ccg cag gac tac gtg gac ccg cac aac gcg gcg cgc gcc 208  
Gln Asn Ser Pro Gln Asp Tyr Val Asp Pro His Asn Ala Ala Arg Ala  
25 30 35

gac gtg ggc gtc ggg ccg gtg tcc tgg gac gac acc gtc gcc gcg tac 256  
Asp Val Gly Val Gly Pro Val Ser Trp Asp Asp Thr Val Ala Ala Tyr  
40 45 50 55

gcg cag agc tac gcg gcg cag cgc cag ggc gac tgc cag ctg atc cac 304  
Ala Gln Ser Tyr Ala Ala Gln Arg Gln Gly Asp Cys Gln Leu Ile His  
60 65 70

WO 99/43819

PCT/US99/03011

tcc ggc ggg ccc tac ggc gag aac ctc ttc tgg ggc tcc gcc ggc gcc 352  
 Ser Gly Gly Pro Tyr Gly Glu Asn Leu Phe Trp Gly Ser Ala Gly Ala  
                   75                                  80                                  85

gac tgg tcg gcg tcc gac gcc gtg ggc tcc tgg gtg tcc gag aag cag 400  
 Asp Trp Ser Ala Ser Asp Ala Val Gly Ser Trp Val Ser Glu Lys Gln  
                   90                                  95                                  100

tac tac gac cac gac acc aac agc tgc gcg gag ggg cag gtg tgc ggc 448  
 Tyr Tyr Asp His Asp Thr Asn Ser Cys Ala Glu Gly Gln Val Cys Gly  
                   105                                  110                                  115

cac tac acg cag gtg gtg tgg cgc gac tcc acc gcc atc ggc tgt gcc 496  
 His Tyr Thr Gln Val Val Trp Arg Asp Ser Thr Ala Ile Gly Cys Ala  
                   120                                  125                                  130                                  135

cgc gtc gtc tgc gac aac aac gcc ggc gtc ttc atc atc tgc agc tac 544  
 Arg Val Val Cys Asp Asn Asn Ala Gly Val Phe Ile Ile Cys Ser Tyr  
                                   140                                  145                                  150

aac ccg ccg ggc aac gtc gtc ggc gag agc ccc tac tag actgtcatgc 593  
 Asn Pro Pro Gly Asn Val Val Gly Glu Ser Pro Tyr  
                   155                                  160

atactacaat tatatatatta tatacgctta atattaattt cagcatgcat gcatattata 653

aatagtgttg tcaactctgt atatcattat attacgatga ttatattggt gatgaacata 713

ataagtcaat aaaaccatgc aggcgatttg tgagcc 749

<210> 13  
 <211> 163  
 <212> PRT  
 <213> Zea mays

<400> 13  
 Met Ala Pro Arg Leu Ala Cys Leu Leu Ala Leu Ala Met Ala Ala Ile  
           1                                  5                                  10                                  15

Val Val Ala Pro Cys Thr Ala Gln Asn Ser Pro Gln Asp Tyr Val Asp  
                   20                                  25                                  30

Pro His Asn Ala Ala Arg Ala Asp Val Gly Val Gly Pro Val Ser Trp  
                   35                                  40                                  45

Asp Asp Thr Val Ala Ala Tyr Ala Gln Ser Tyr Ala Ala Gln Arg Gln



WO 99/43819

PCT/US99/03011

gtg gcg tgg gac gcc agg gtg gcc agg tac gcg cag gac tac gcg gcg 248  
 Val Ala Trp Asp Ala Arg Val Ala Arg Tyr Ala Gln Asp Tyr Ala Ala  
 55 60 65

aag cgc gcc ggg gac tgc cgg ctg gtg cac tcg ggc ggg ccg ttc ggc 296  
 Lys Arg Ala Gly Asp Cys Arg Leu Val His Ser Gly Gly Pro Phe Gly  
 70 75 80 85

gag agc atc ttc tgg ggc tcg gcg ggg cgg gcg tgg agc gcc gcc gac 344  
 Glu Ser Ile Phe Trp Gly Ser Ala Gly Arg Ala Trp Ser Ala Ala Asp  
 90 95 100

gcg ctg cgg tcg tgg gtg gac gag aag agg aac tac cac ctg agc agc 392  
 Ala Leu Arg Ser Trp Val Asp Glu Lys Arg Asn Tyr His Leu Ser Ser  
 105 110 115

aac acc tgc gac ccc ggc aag gtg tgc ggc cac tac acg cag gtg gtg 440  
 Asn Thr Cys Asp Pro Gly Lys Val Cys Gly His Tyr Thr Gln Val Val  
 120 125 130

tgg cgc agg tgt cca ccc gca tcg gct gcg cgc gcg tcg tct gcg ccg 488  
 Trp Arg Arg Cys Pro Pro Ala Ser Ala Ala Arg Ala Ser Ser Ala Pro  
 135 140 145

aca acc gcg gcg tct tca tcg tct gca gct acg acc ccc cgg gca acg 536  
 Thr Thr Ala Ala Ser Ser Ser Ser Ala Ala Thr Thr Pro Arg Ala Thr  
 150 155 160 165

tca acg gcc agc gcc cgt tcc tca ctc tcg acg cgg ctg cca agt aga 584  
 Ser Thr Ala Ser Ala Arg Ser Ser Leu Ser Thr Arg Leu Pro Ser Arg  
 170 175 180

ggc aga gag ccc ggc tgc atg cag tgt gcg tac gca cgc atc tgc gtg 632  
 Gly Arg Glu Pro Gly Cys Met Gln Cys Ala Tyr Ala Arg Ile Cys Val  
 185 190 195

tgc atg gcg tgg cta ctc gat cga tca cgt act gcg tgt gcg cgc gca 680  
 Cys Met Ala Trp Leu Leu Asp Arg Ser Arg Thr Ala Cys Ala Arg Ala  
 200 205 210

cca taa taagtattgt gtgtacgtat atatctgcat ctgcagtgtt tgtgtcatat 736  
 Pro  
 215

ataaaataat cgtctgcgtg cgctatataa tatctatana acttcaataa ttttacataa 796

aaaaaaaaaa 806

WO 99/43819

PCT/US99/03011

&lt;210&gt; 15

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Zea mays

&lt;400&gt; 15

Met Ala Phe Pro Lys Pro Thr Ser Arg Leu Ala Ala Leu Ala Ala Leu  
 1 5 10 15

Ala Ala Ala Met Ala Ala Ala Met Met Ala Ala Thr Ala Ser Ala Gln  
 20 25 30

Asn Thr Pro Gln Asp Phe Val Asn Leu His Asn Arg Ala Arg Ala Ala  
 35 40 45

Asp Gly Val Gly Pro Val Ala Trp Asp Ala Arg Val Ala Arg Tyr Ala  
 50 55 60

Gln Asp Tyr Ala Ala Lys Arg Ala Gly Asp Cys Arg Leu Val His Ser  
 65 70 75 80

Gly Gly Pro Phe Gly Glu Ser Ile Phe Trp Gly Ser Ala Gly Arg Ala  
 85 90 95

Trp Ser Ala Ala Asp Ala Leu Arg Ser Trp Val Asp Glu Lys Arg Asn  
 100 105 110

Tyr His Leu Ser Ser Asn Thr Cys Asp Pro Gly Lys Val Cys Gly His  
 115 120 125

Tyr Thr Gln Val Val Trp Arg Arg Cys Pro Pro Ala Ser Ala Ala Arg  
 130 135 140

Ala Ser Ser Ala Pro Thr Thr Ala Ala Ser Ser Ser Ser Ala Ala Thr  
 145 150 155 160

Thr Pro Arg Ala Thr Ser Thr Ala Ser Ala Arg Ser Ser Leu Ser Thr  
 165 170 175

Arg Leu Pro Ser Arg Gly Arg Glu Pro Gly Cys Met Gln Cys Ala Tyr  
 180 185 190

Ala Arg Ile Cys Val Cys Met Ala Trp Leu Leu Asp Arg Ser Arg Thr  
 195 200 205

Ala Cys Ala Arg Ala Pro

WO 99/43819

PCT/US99/03011

210

&lt;210&gt; 16

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 16

gtaatacgac tcactatagg gc

22

&lt;210&gt; 17

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 17

agctaaacac acgacgaccc tagtagacga

30

&lt;210&gt; 18

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 18

ctccacctc ggcgcccgcc attacag

27

&lt;210&gt; 19

&lt;211&gt; 28

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Maize

WO 99/43819

PCT/US99/03011

gene-specific PCR primer

&lt;400&gt; 19

gccagtccat cacggcggcg cggaacag

28

&lt;210&gt; 20

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 20

cacgctagcc tcggtgccat tgtttgttgt

30

&lt;210&gt; 21

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 21

ggctagacga ctagtaggct tcggaaa

27

&lt;210&gt; 22

&lt;211&gt; 19

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 22

actatagggc acgcgtggt

19

&lt;210&gt; 23

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

WO 99/43819

PCT/US99/03011

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 23

gcacggcatc acggcgacca tggccaa

27

&lt;210&gt; 24

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 24

gcgcccggcca ttacagcctc ctcgcgtctt

30

&lt;210&gt; 25

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 25

gggctagctg aaggctgaag cagatgg

27

&lt;210&gt; 26

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 26

gacgatggct gccatggcca cagctaggag

30

&lt;210&gt; 27

WO 99/43819

PCT/US99/03011

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 27

ccatggccgc agcgagggca gctagcg

27

&lt;210&gt; 28

&lt;211&gt; 26

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 28

gctaccacac aaaaacttca tatattg

26

&lt;210&gt; 29

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 29

gcatcacggc gaccatggcc aac

23

&lt;210&gt; 30

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 30

aggcggcagc ggccaagacc ggtgt

25

WO 99/43819

PCT/US99/03011

&lt;210&gt; 31

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 31

ggtgtccctc tcgccctcgc cgtgg

25

&lt;210&gt; 32

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 32

cccacgcgtc cggaagctac aatcc

25

&lt;210&gt; 33

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 33

gccaccggcg acgcgatctc gag

23

&lt;210&gt; 34

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 34

WO 99/43819

PCT/US99/03011

cattggactt gcactggtgc ttgc

24

&lt;210&gt; 35

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 35

gcgccacgac gatggctgcc atg

23

&lt;210&gt; 36

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: PCR primer

&lt;400&gt; 36

gaataactaa tcaagatcga tcg

23

&lt;210&gt; 37

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Maize  
gene-specific PCR primer

&lt;400&gt; 37

cgccgaggcg gtcgcggcca tc

22

50

## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule having a nucleotide sequence for a promoter that is capable of initiating transcription in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:
  - 5 a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5;
  - b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No. \_\_\_\_\_;
  - c) a nucleotide sequence comprising at least 40 contiguous  
10 nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and
  - d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).
2. A DNA construct comprising a nucleotide sequence of claim  
15 1 operably linked to a heterologous nucleotide sequence of interest.
3. A vector comprising the DNA construct of claim 2.
4. A host cell having stably incorporated in its genome the DNA  
20 construct of claim 2.
5. A method for inducing expression of a heterologous nucleotide sequence in a plant, said method comprising transforming a plant cell with a DNA construct comprising said heterologous nucleotide sequence operably linked  
25 to a promoter that is capable of initiating transcription in a plant cell in response to a stimulus, regenerating a stably transformed plant from said plant cell, and exposing said plant to said stimulus, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:
  - a) a nucleotide sequence comprising the sequence set forth in  
30 SEQ ID NO: 1, 2, 3, or 4;

b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No. \_\_\_\_\_;

5 c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, or 4; and

d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

10 6. The method of claim 5, wherein said plant is a monocot.

7. The method of claim 6, wherein said monocot is maize.

8. The method of claim 6, wherein said plant is a dicot.

15 9. A method for constitutively expressing a heterologous nucleotide sequence in a plant, said method comprising transforming a plant cell with a DNA construct comprising said heterologous nucleotide sequence operably linked to a promoter that is capable of initiating constitutive transcription in a plant cell and regenerating a stably transformed plant from said plant cell, wherein said  
20 promoter comprises a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 5;

b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No. \_\_\_\_\_;

25 c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 5; and

d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

30 10. The method of claim 9, wherein said plant is a monocot.

11. The method of claim 10, wherein said monocot is maize.

12. The method of claim 9, wherein said plant is a dicot.

13. A plant cell stably transformed with a DNA construct comprising  
5 a heterologous nucleotide sequence operably linked to a promoter that is capable  
of initiating transcription in said plant cell, wherein said promoter comprises a  
nucleotide sequence selected from the group consisting of :

- a) a nucleotide sequence comprising the sequence set forth in  
SEQ ID NO: 1, 2, 3, 4, or 5;
- 10 b) a nucleotide sequence selected from the group consisting of  
sequences deposited as ATCC Accession No. \_\_\_\_\_;
- c) a nucleotide sequence comprising at least 40 contiguous  
nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and
- d) a nucleotide sequence that hybridizes under stringent  
15 conditions to a sequence of a), b), or c).

14. The plant cell of claim 13, wherein said plant cell is from a  
monocot.

20 15. The plant cell of claim 14, wherein said monocot is maize.

16. The plant of claim 13, wherein said plant cell is from a dicot.

17. A plant stably transformed with a DNA construct comprising a  
25 heterologous nucleotide sequence operably linked to a promoter that is capable of  
initiating transcription in a plant cell, wherein said promoter comprises a  
nucleotide sequence selected from the group consisting of:

- a) a nucleotide sequence comprising the sequence set forth in  
SEQ ID NO: 1, 2, 3, 4, or 5;
- 30 b) a nucleotide sequence selected from the group consisting of  
sequences deposited as ATCC Accession No. \_\_\_\_\_;

c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and

d) a nucleotide sequence that hybridizes under stringent  
5 conditions to a sequence of a), b), or c).

18. The plant of claim 17, wherein said plant is a monocot.

19. The plant of claim 18, wherein said monocot is maize.

10

20. The plant of claim 17, wherein said plant is a dicot.

21. Seed of the plant of any one of claims 17-20.

15 22. An isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;

20 b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No. \_\_\_\_\_;

c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;

d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and

25 e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

23. A DNA construct comprising a nucleotide sequence of claim 22 operably linked to a promoter that drives expression in a plant cell.

30

24. A vector comprising the DNA construct of claim 23.

25. A host cell having stably incorporated in its genome the DNA construct of claim 23.

5 26. A method for creating or enhancing disease resistance in a plant, said method comprising transforming said plant with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell and regenerating stably transformed plants, wherein said PR-1 sequence is selected from the group consisting of:

10 a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;

b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No. \_\_\_\_\_;

15 c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;

d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and

e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

20 27. The method of claim 26, wherein said plant is a monocot.

28. The method of claim 27, wherein said monocot is maize.

25 29. The method of claim 26, wherein said promoter is a constitutive promoter.

30 30. The method of claim 29, wherein said constitutive promoter is the promoter for PR-1#93.

31. The method of claim 26, wherein said promoter is an inducible promoter.

5 32. The method of claim 31, wherein said inducible promoter is selected from the group consisting of promoters for PR-1#52, PR-1#71, PR-1#81, and PR-1#83.

33. A plant cell stably transformed with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding  
10 sequence in a plant cell, wherein said PR-1 sequence is selected from the group consisting of:

- a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
- b) a nucleotide sequence selected from the group consisting of  
15 sequences deposited as ATCC Accession No. \_\_\_\_\_;
- c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: : 7, 9, 11, or 15;
- d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and
- 20 e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

34. A plant stably transformed with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding  
25 sequence in a plant cell, wherein said PR-1 sequence is selected from the group consisting of:

- a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
- b) a nucleotide sequence selected from the group consisting of  
30 sequences deposited as ATCC Accession No. \_\_\_\_\_;
- c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: : 7, 9, 11, or 15;

WO 99/43819

PCT/US99/03011

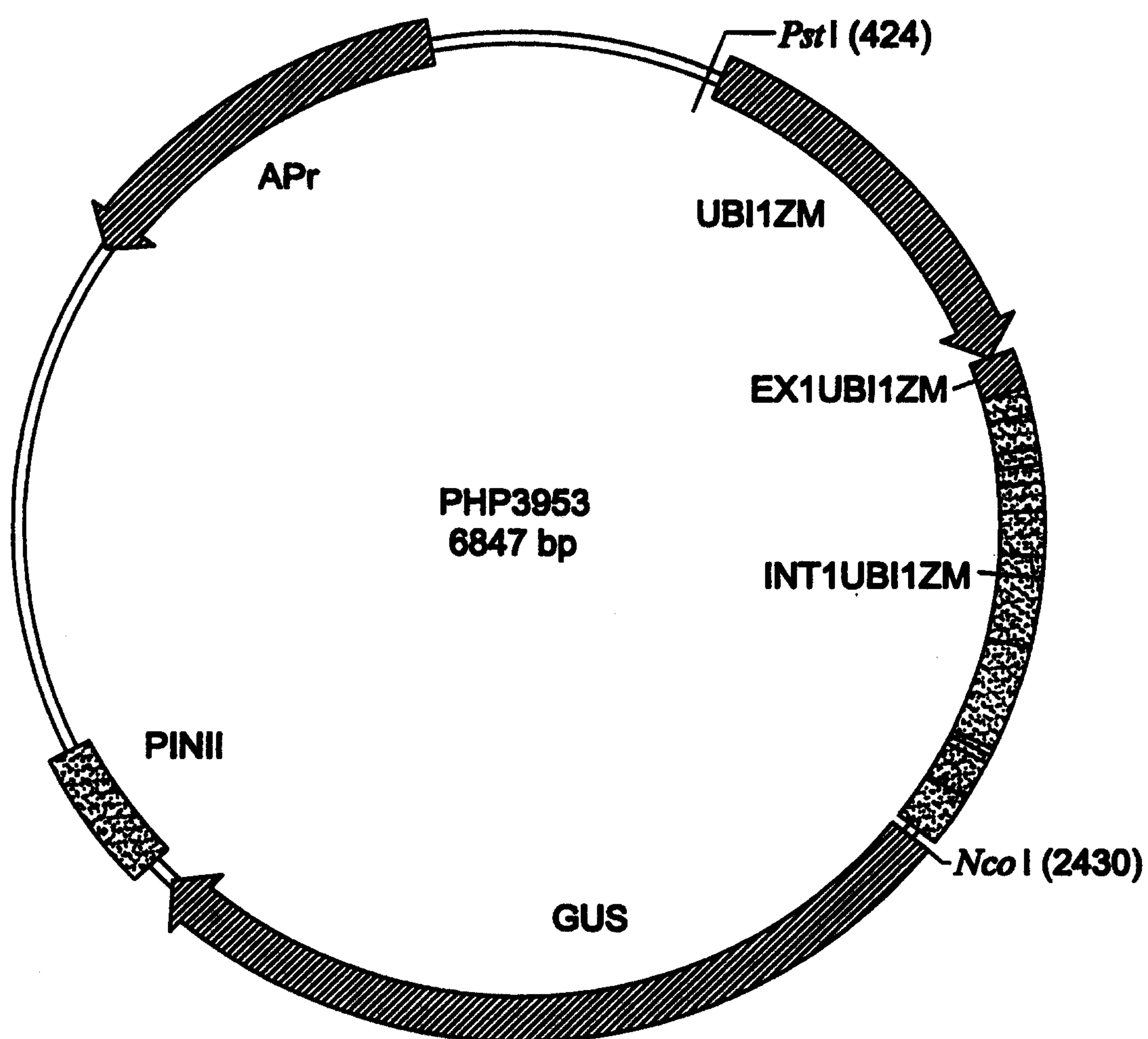
56

d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and

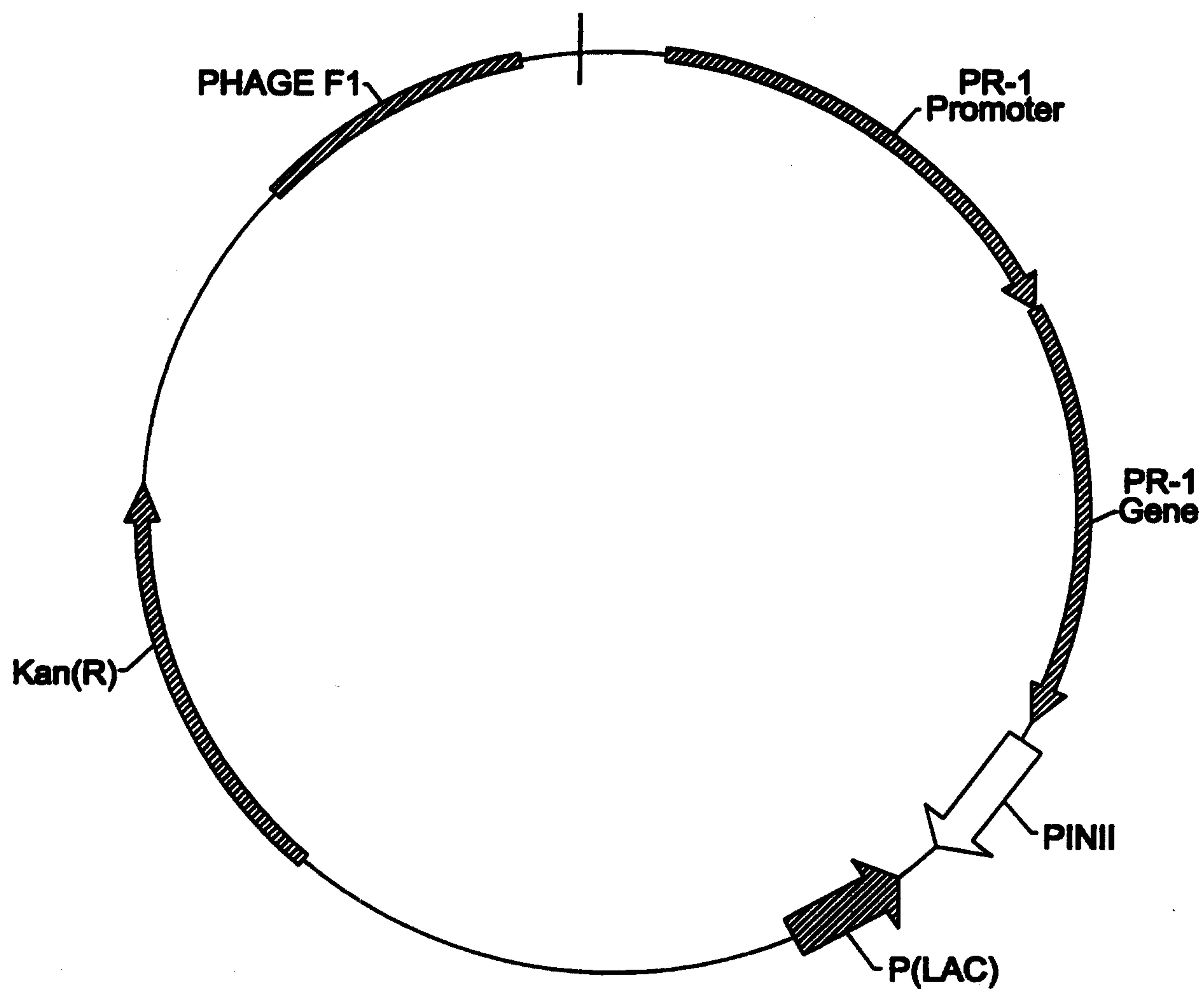
e) a nucleotide sequence that hybridizes under stringent  
5 conditions to a sequence of a), b), c), or d).

35. Seed of the plant of claim 34.

1/2

FIG. 1.

2/2

FIG. 2.

