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(54) Title: COMPOUNDS USEFUL TO AFFECT RESISTANCE IN PLANTS AND METHODS RELATED THERETO

(57) Abstract

The present invention provides materials and methods related to causing disease resistance in plants. Specifically, the present invention provides nucleic acid compounds which encode an avirulence gene product which is capable of causing a hypersensitive response in plants which recognize the gene product. A nucleic acid compound specifically set forth in the sequence listing is an avirulence gene from Xanthomonas oryzae pv. oryzae, a rice pathogen) which causes a resistance response in maize. Cells and plants (including plant parts, seeds, embryos, etc.) comprising the nucleic acid compounds are also within the scope of the present invention. The present invention also provides compounds encoding the avirulence gene products encoded by the nucleic acid compounds of the present invention, as well as methods to induce resistance, methods to cause cell death, and methods to affect other plant cellular processes.
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Compounds Useful to Affect Resistance in
Plants and Methods Related Thereto

This application claims priority to provisional patent application
serial number 60/096,585, filed on August 14, 1998.

BACKGROUND OF THE INVENTION

Plants and plant pathogens possess elegant molecular systems for
interacting with each other. Pathogens with traits which make them
comparatively superior at infecting plants have survived; likewise,
plants with traits which make them comparatively superior at resisting
infection have survived. The molecular processes which have been
selected by these forces over time are quite interwoven and intricate.

For example, some pathogens carry genes that express gene
products useful to gain entry into the plant. Some plants, however, have
the ability to “recognize” these or other pathogen gene products and use
the information to combat the pathogen. The plants able to recognize the
pathogen gene product are stimulated to actively resist the pathogen.
One means is to produce a physical barrier to the pathogen, either by the
production of wall materials and/or by a localized breakdown of plant
cellular material (or plant cell death, called the hypersensitive response
or HR). In broad terms, such a plant is capable of mounting an
“immune response” to the “antigen” presented by the pathogen.

The pathogen genes described above have been called “avirulence”
genes, primarily because their presence in the pathogen prevents
pathogen multiplication and spread if the plant contains the
corresponding resistance gene. This type of plant defense (antigen
recognition) function has been well documented. A thorough review of
bacterial avirulence genes was made in 1996 by Drs. Leach and White.
In their review, Leach and White compare the known sequences of avirulence genes and remark that the various sequences are relatively dissimilar, generally encode a single open reading frame, and do not contain recognizable motifs as ie. kinases or export signals. With regard to a certain sub-group of avirulence genes, the *avrBs3* family, the authors comment that “the central domain of these genes contains a series of 102 bp, directly repeated DNA sequences.” Leach and White, p. 157 (1996). The number of 102 bp repeats was shown to be different among members of the *avrBs3* family. *ibid.* Also, minor differences were shown to occur within the 102 bp repeats of a given gene, with most of the differences located within a variable two codon region. *ibid.* Of relevance to this document, members of the *avrBs3* gene family, specifically *avrXa10* and *avrXa7*, have been cloned from a bacterial rice pathogen, *Xanthomonas oryzae* pv. *oryzae*, and have been shown to function as avirulence genes (Hopkins *et al.*, Molecular Plant-Microbe Interactions, 1992, 5:451-459).

Table 1 on pages 158 and 159 of Leach and White shows the ability of some avirulence genes to cause a hypersensitive response on more than one plant species or cultivar. Leach and White also comment that a particular avirulence gene, *avrRxv* “is broadly recognized and not only conferred avirulence to *X. campestris* pv. *phaseoli* for particular bean cultivars, but also resulted in induction of resistance by several *X. campestris* pathovars on their normally susceptible hosts (pv. *glycines* on soybean, pv. *vignicola* on cowpea, pv. *holcicola* on corn, pv. *alfalfae* on alfalfa and pv. *malvacearum* on cotton).” Leach and White, p. 155 (1996). Leach and White do not make broad generalizations, however. Leach and White, p. 156 (1996).

Citation of the above documents is not intended as an admission that any of the foregoing is pertinent prior art. For example, in some instances above, the publication was less than one year before the filing date of this patent application. All statements as to the date or representation as to the contents of these documents is based on subjective characterization of information available to the applicant at
the time of filing, and does not constitute an admission as to the accuracy of the dates or contents of these documents.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1. The vector pCH43, which is a high copy plasmid (based on pBluescript) modified so that the BamHI fragment, which contains most of the coding region, or the SphI fragment, which contains the repeat from members of the avrXa10 gene family, can be inserted between the conserved 5' and 3' regions to test for gene function (avirulence activity). This may be used as an expression system to simplify identification of functional avirulence genes and to determine if the repeat domain confers host specificity in this gene family.

**SUMMARY OF THE INVENTION**

It is therefore an object of the present invention to provide nucleic acids useful to engineer inducible resistance in plants.

It is a further object to provide methods to engineer inducible resistance in plants.

It is yet another object to provide seeds, plant embryos, plants and plant parts capable of inducible resistance.

It is also an object of the invention to provide materials such as vectors and cells for genetic engineering of inducible resistance lines.

It is an additional object to provide methods to improve genetic manipulations of plants, such as flower loss and fruit harvest, using the materials and methods herein disclosed.

In all of the above embodiments, it is an object to provide materials and methods useful in scientific research.
In a particular embodiment, it is an object to provide a rice pathogen avirulence gene and gene product which gene product is recognized by maize lines which carry the corresponding resistance gene.

Lastly, it is therefore an object of the present invention to provide materials and methods useful to cause a plant to evoke a hypersensitive response when presented with a predetermined environmental or internal stimulus.

Definitions:

For the purposes of the present application, the following terms have the following meanings. All other terms have the meaning as generally recognized in the art.

"Allelic variant" is meant to refer to a gene that occurs at essentially the same locus (or loci) as the referent sequence, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions).

"Fragment" is meant to refer to a nucleic acid or polypeptide subset of the referent compound, wherein the referent subset has at least 300, at least 500 and preferably at least 1000 contiguous bases of the referent compound.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides materials and methods related to causing resistance in plants. Specifically, the present invention provides nucleic acid compounds that encode an avirulence gene
product which is capable of causing a hypersensitive response in plants which recognize the gene product. A nucleic acid compound specifically set forth in the sequence listing is an avirulence gene from *Xanthomonas oryzae pv. oryzicola* (a rice pathogen) which causes a resistance response in maize. The nucleic acid compounds include fragments and complements of the sequences in the sequence listing, as well as sequences which are easily obtained by virtue of the knowledge of these sequences. The nucleic acid compounds herein provided also include variations on the fragments, complements and easily-obtained sequences, such as vectors or other constructs containing the fragments, complements and easily-obtained sequences. Cells and plants (including plant parts, seeds, embryos, etc.) comprising the nucleic acid compounds are also within the scope of the present invention.

The present invention also provides compounds encoding the avirulence gene products encoded by the nucleic acid compounds of the present invention, as well as methods to induce avirulence, methods to cause cell death, and methods to affect other cellular processes.

It is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCG™ (available from Genetics Computer Group, Madison, WI), DNAsis™ (available from Hitachi Software, San Bruno, CA) and MacVector™ (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Compare function by maximum matching within the program DNAsis Version 2.1 using
default parameters. A nucleic acid sequence of the present invention may have at least 85%, preferably 90%, and most preferably 95% sequence identity with a nucleic acid molecule in the sequence listing.

Therefore, the present invention provides isolated nucleic acid compounds, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence which has at least 95% identity to a nucleic acid which is selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; wherein said identity can be determined using the DNAsis computer program and default parameters;

(b) a nucleic acid sequence which encodes an amino acid sequence which has at least 95% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters;

(c) a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; and

(d) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); and a nucleic acid sequence of (c).

Preferred are isolated nucleic acid molecules, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1; and SEQ ID NO 3;
(b) a nucleic acid sequence which encodes an amino acid sequence
selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6;

(c) a nucleic acid sequence which is an allelic variant of a nucleic
acid sequence selected from the group consisting of: SEQ ID NO 1; and
SEQ ID NO 3; and

(d) a nucleic acid sequence fully complementary to a nucleic acid
sequence selected from the group consisting of: a nucleic acid sequence
of (a); a nucleic acid sequence of (b); and a nucleic acid sequence of (c).

The present invention also provides isolated nucleic acid
compounds comprising a nucleic acid compound which hybridizes
under stringent conditions to SEQ ID NO 1 or SEQ ID NO 3.

Stringent hybridization conditions are determined based on
defined physical properties of the gene to which the nucleic acid
molecule is being hybridized, and can be defined mathematically.
Stringent hybridization conditions are those experimental parameters
that allow an individual skilled in the art to identify significant
similarities between heterologous nucleic acid molecules. These
conditions are well known to those skilled in the art. See, for example,
138, 267-284, each of which is incorporated by reference herein in its
entirety. As explained in detail in the cited references, the
determination of hybridization conditions involves the manipulation of a
set of variables including the ionic strength (M, in moles/liter), the
hybridization temperature (°C), the concentration of nucleic acid helix
destabilizing agents (such as formamide), the average length of the
shortest hybrid duplex (n), and the percent G+C composition of the
fragment to which an unknown nucleic acid molecule is being
hybridized. For nucleic acid molecules of at least about 150 nucleotides,
these variables are inserted into a standard mathematical formula to
calculate the melting temperature, or Tm, of a given nucleic acid molecule. As defined in the formula below, Tm is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands: 

\[ Tm = 81.5^\circ C + 16.6 \log M + 0.41(\%G+C) - 500/n 0.61(\% formamide) \]

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (Td), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation: 

\[ Td = 4(G+C) + 2(A+T) \]

A temperature of 5^\circ C below Td is used to detect hybridization between perfectly matched molecules.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

Also included in the scope of the present invention is an isolated nucleic acid compound which is an approximately 3.7 kb fragment isolated from *Xanthomonas oryzae pv. oryzicola* BLS 256, and which fragment hybridizes to *avrXa10*, an avirulence gene cloned from the related pathogen *X. oryzae pv. oryzae* PXO86 (Hopkins *et al.*, Molecular Plant-Microbe Interactions 5:451-459).

Also included in the scope of the present invention are isolated nucleic acid molecules, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:
(a) a nucleic acid comprising at least 2000 contiguous nucleotides of SEQ ID NO 1;

(b) a nucleic acid comprising at least 1000 contiguous nucleotides of SEQ ID NO 3;

(c) a nucleic acid which encodes an amino acid comprising at least 900 contiguous residues of SEQ ID NO 5;

(d) a nucleic acid which encodes an amino acid comprising at least 450 contiguous residues of SEQ ID NO 6;

(e) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); a nucleic acid sequence of (c); and a nucleic acid sequence of (d).

Preferred embodiments of the above are isolated nucleic acid molecules, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid comprising at least 2500 contiguous nucleotides of SEQ ID NO 1;

(b) a nucleic acid comprising at least 1500 contiguous nucleotides of SEQ ID NO 3;

(c) a nucleic acid which encodes an amino acid comprising at least 1250 contiguous residues of SEQ ID NO 5;

(d) a nucleic acid which encodes an amino acid comprising at least 750 contiguous residues of SEQ ID NO 6;
(e) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); a nucleic acid sequence of (c); and a nucleic acid sequence of (d).

Included within the scope of the present invention, with particular regard to the nucleic acids above, are allelic variants, degenerate sequences and homologues. Allelic variants are well known to those skilled in the art and would be expected to be found within a given plant or microbe and/or among a group of two or more plants or microbes. The present invention also includes variants of *aurRxo1* due to laboratory manipulation, such as, but not limited to, variants produced during polymerase chain reaction amplification or site directed mutagenesis. It is also well known that there is a substantial amount of redundancy in the various codons which code for specific amino acids. Moreover, variants of the universal code, such as are present in some plant, animal and fungal mitochondria, the bacterium *Mycoplasma capricolum* (82 Proc Natl Acad Sci (USA) 2306 (1985)) or the ciliate *Macronucleus*, may be used when the nucleic acid is expressed using these organisms. Therefore, this invention is also directed to those nucleic acid sequences which contain alternative codons which code for the eventual translation of the amino acid.

Also included within the scope of this invention are mutations either in the nucleic acid sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide. Lastly, a nucleic acid sequence homologous to the exemplified nucleic acid compounds (or allelic variants or degenerates thereof) will have at least 70%, preferably 80%, and most preferably 90% sequence homology with the nucleic acid compounds in the sequence listing. Most preferred is an mRNA which is complementary to the DNA compounds in the sequence listing. In other words, a nucleic acid sequence homologous to a nucleic acid is
characterized by the ability to hybridize to the exemplified nucleic acid compounds (or allelic variants or degenerates thereof) under stringent conditions. Stringent hybridization conditions are described in Sambrook et al., *Molecular Cloning. A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989).

A variety of procedures known in the art may be used to molecularly clone the present nucleic acids. These methods include, but are not limited to complementation for function following the construction of a genomic DNA library in an appropriate vector system. Another method is to screen a genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled oligonucleotide probe designed from the amino acid sequence of the gene. An additional method consists of screening genomic DNA libraries constructed in a bacteriophage or plasmid shuttle vector with a partial DNA encoding the gene. This partial DNA is obtained by specific PCR amplification of the gene DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence of the purified gene product or by using another member of the gene family as a probe. Sambrook et al., *Molecular Cloning. A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989) and Ausubel et al., *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc., 1993) describe these procedures. Alternatively, the nucleic acids can be prepared as exemplified herein.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ. (Murray et al., *17 Nucl Acids Res 477* (1989). Thus, the maize preferred codon for a particular amino acid may be derived
from known gene sequences from maize. Maize codon usage for 28
genomes from maize plants are listed in Table 4 of Murray et al., supra.

The cloned nucleic acids may be expressed through the methods
described in the examples or methods known in the art. The DNA can
be recombinantly expressed by molecular cloning into an expression
vector containing a suitable promoter and other appropriate
transcription regulatory elements, and transferred into prokaryotic or
eukaryotic host cells to produce recombinant gene product. Techniques
for such manipulations are fully described in Sambrook, J., et al, supra.
Expression vectors can be used to express genes in a variety of hosts
such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells
and animal cells. Expression vectors may include, but are not limited
to, cloning vectors, modified cloning vectors, specifically designed
plasmids or viruses.

Vectors which comprise the nucleic acid compounds are within
the scope of the present invention, as are plants transformed with the
above nucleic acid compounds. Vectors may be obtained from various
commercial sources, including Clontech Laboratories, Inc. (Palo Alto,
CA), Stratagene (La Jolla, CA), Invitrogen (Carlsbad, CA), New
England Biolabs (Beverly, MA) and Promega (Madison, WI).

Preferred vectors are those which are capable of transferring the
sequences disclosed herein into plant cells or plant parts. Expression
vectors are preferred, with expression vectors comprising an inducible
promoter operably linked to the nucleic acid compound being more
preferred. "Inducible" promoters typically direct expression of a
polynucleotide in a specific tissue or may be otherwise under more
precise environmental or developmental control. The most preferred
vectors herein provided are expression vectors comprising a tightly-
regulated inducible promoter operably linked to the nucleic acid
compound. Also included is a vector which further comprises the plant
resistance gene either operably linked to the tightly-regulated inducible
promoter, or operably linked to a second, tightly-regulated inducible
promoter.
Environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions, or the presence of light. Examples of inducible promoters are the Adh1 promoter which is inducible by hypoxia or cold stress, the Hsp70 promoter which is inducible by heat stress, and the PPDK promoter which is inducible by light. Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. An exemplary promoter is the anther specific promoter 5126 (US Patent Nos 5,689,049 and 5,689,051). The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. When the genes are expressed at levels to cause cell death, an inducible promoter can be used to drive expression of either the avirulence or the resistance gene, or both genes. Where the resistance gene is present in the plant or is crossed into the plant through breeding methods, the avirulence gene can be expressed utilizing an inducible promoter. The inducible promoter is ideally tightly regulated so as to prevent unnecessary cell death, yet be expressed in the presence of a pathogen to prevent infection and/or disease symptoms. Generally, it will be beneficial to express the gene from a pathogen-inducible promoter. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen, e.g. PR proteins, SAR proteins, beta-1,3 glucanase, chitinase, etc. See, for example, Redolfi et al., 89 Neth J Plant Pathol 245 (1983); Ukenes et al., 4 Plant Cell 645 (1992); Van Loon, 4 Plant Mol Virol 111 (1985).

Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau et al., 9 Plant Mol Biol 335 (1987); Matton et al., 2 Mol Plant-Microbe Interact 325 (1989); Somsisch et al., 2 Mol and Gen Genetics 93 (1988). Yang, 93 Proc Natl
Acad Sci 14972 (1996). See also, Chen et al., 10 Plant J 955 (1996); Zhang and Sing, 91 Proc Natl Acad Sci USA 2507 (1994); Warner et al., 3 Plant J 191 (1993); Siebertz et al., 1 Plant Cell 961 (1989); 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., 41 Physiol and Mol Plant Path 189 (1992).

For use in experimental systems, inducible expression of the avr gene from chemically-inducible promoters, such as the DEX promoter (Aoyama and Chua, 1997, Plant Journal, 11:605-612), will avoid lethality problems resulting from expression of the avr gene in host plants carrying the complementary resistance gene (recognition factor).

Additionally, because pathogens find entry into plants through wounds or insect damage, a wound-inducible promoter may be used in the constructions of the invention. Such wound inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan, 28 Annu Rev Phytopath 425 (1990); Duan et al., 14 Nature Biotech 494 (1996); wun1 and wun2, US Patent Serial Number 5,428,148; win1 and win2 (Stanford et al., 215 Mol Gen Genet 200 (1989); systemin (McGurl et al., 225 Science 1570 (1992); WIPI (Rohrmeier et al., 22 Plant Mol Biol 783 (1993); Eckelkamp et al., 323 FEBS Let 73 (1993); MPI gene (Cordero et al., 6(2) Plant J 141 (1994) and references contained therein.

Rice peroxidase promoters that are (1) wound-inducible (2) pathogen- and wound- inducible, or (3) pathogen-inducible are particularly useful in the present invention, and are within the scope of the present claims. Maize promoters as described in the previous paragraphs are also preferred for use in the present vectors. Inducible promoters can either be constructed de novo according to known techniques, or obtained from various commercial sources, including those described above for obtaining vectors. Construction of vectors comprising promoters in frame with nucleic acids is known in the art, and can be accomplished according to i.e. Sambrook et al., Molecular

Also included in the present invention are recombinant plant cells, recombinant seeds, recombinant plant embryos and recombinant plants comprising the vectors described herein. Monocotyledons comprising the vectors described herein are preferred. However, Xanthomonas oryzae pv. oryzicola BLS 256 comprising an isolated nucleic acid compound which is an approximately 3.7 kb fragment, and which hybridizes under stringent conditions to avrXa10 is preferred. Xanthomonas oryzae pv. oryzae PXO99A comprising the an approximately 3.7 kb fragment, which fragment hybridizes to avrXa10 (and which incites a strong hypersensitive response on a particular maize cultivar) is also preferred.

For example, the following seeds, embryos, plants or plant parts transformed with herein-disclosed nucleic acid constructs are considered within the present invention: rice; soybean; maize; beet; tobacco; wheat; barley; poppy; rape; sunflower; alfalfa; sorghum; rose; carnation; gerbera; carrot; tomato; lettuce; chicory; pepper; melon; cabbage; canola; tulip; orchid and lily. Particularly preferred are: sorghum; soybean; sunflower; canola; alfalfa; rice; corn; wheat; cotton and barley. Seeds, embryos, plants or plant parts which naturally mount a hypersensitive response to the present amino acid compound(s) and comprise herein-disclosed constructs are preferred embodiments of the present invention. Of course, those in the art recognize that any seed, embryo or plant transformed with the present constructs which are useful for producing plants for biomass are within the scope of the present invention.
Transformation of cells with the nucleic acid compounds of the present invention can be accomplished according to known procedures. For example, infective, vector-containing bacterial strains (such as Agrobacterium rhizogenes and Agrobacterium tumefaciens) may be used for transformation. Zambryski, 43 Ann. Rev. Pl. Physiol. Pl. Mol. Biol. 465 (1992). The following procedures are also well-known: Pollen-tube transformation [Zhou-xun et al., 6 Plant Molec. Bio. 165 (1988)]; direct transformation of germinating seeds [Toepfer et al., 1 Plant Cell 133 (1989)]; polyethylene glycol or electroporation transformation [Christou et al., 84 Proc. Nat. Acad. Sci. 3662 (1987)]; and biolistic processes [Yang & Cristou, Particle Bombardment Technology for Gene Transfer (1994)]. The transformed cells are also within the scope of the present invention.

The transformed cells may be induced to form transformed plants via organogenesis or embryogenesis, according to the procedures of Dixon Plant Cell Culture: A Practical Approach (IRL Press, Oxford 1987).

Also provided are methods for constructing sequences with the ability to knockout the above sequences, comprising one of the following techniques: inserting a foreign piece of DNA into one of the disclosed sequences; deleting a piece of DNA from one of the disclosed sequences; rearranging the sequences (e.g. the repeats); or creating a mutation such that the avirulence activity is eliminated.

Also provided are antisense constructs and methods to inhibit mRNA transcripts of the disclosed sequences, so as to either eliminate or reduce the amount of gene product. The procedures for antisense inhibition for mRNA are described in US Patent 5,554,743, which patent is expressly incorporated by reference into this application.

Another aspect of the present invention are isolated avrRxo amino acid compounds, wherein said amino acid compound comprises an amino acid sequence selected from the group consisting of:
(a) an amino acid sequence which is encoded by a nucleic acid sequence which has at least 95% identity to a nucleic acid which is selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; wherein said identity can be determined using the DNAsis computer program and default parameters;

(b) an amino acid sequence which has at least 95% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters; and

(c) an amino acid sequence which is encoded by a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3.

Preferred are isolated avrRxo amino acid compounds, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; and

(b) an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters;

Also provided are isolated avrRxo amino acid compounds, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid comprising at least 2000 contiguous nucleotides of SEQ ID NO 1;
(b) an amino acid sequence which is encoded by a nucleic acid comprising at least 1000 contiguous nucleotides of SEQ ID NO 3;

(c) an amino acid comprising at least 900 contiguous residues of SEQ ID NO 5; and

(d) a nucleic acid which encodes an amino acid comprising at least 450 contiguous residues of SEQ ID NO 6;

Preferred are isolated avrRxo amino acid compounds, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid comprising at least 2500 contiguous nucleotides of SEQ ID NO 1;

(b) an amino acid sequence which is encoded by a nucleic acid comprising at least 1500 contiguous nucleotides of SEQ ID NO 3;

(c) an amino acid comprising at least 1250 contiguous residues of SEQ ID NO 5; and

(d) an amino acid comprising at least 750 contiguous residues of SEQ ID NO 6.

Modifications of the amino acid compounds, such as conservative changes in the amino acid sequence, or modifications useful to identify existence or location of gene product are also within the scope of the present invention. For example, an engineered antibody recognition site would be helpful for research purposes, or for quality-control in a commercial plant. Such modifications can be accomplished according to Young et al., *9 Mol Plant-Microb Interact* 105 (1994).

The amino acid compounds of the present invention can be purified according to common purification techniques, such as that
described in Bollag et al., Protein Methods (Wiley-Liss 1996); Scopes, Protein Purification: principles and practice (Springe-Verlag 1994); Doonan, Protein Purification Protocols (Humana Press 1996). For example, artisans will also recognize that these compounds (or portions thereof) can be synthesized by solid phase peptide synthesis or recombinant DNA methods.

For purification via recombinant DNA methods, following expression of the nucleic acids disclosed above in a recombinant host cell, the amino acid compounds described herein can be recovered in purified form. Several purification procedures are available and suitable for use. For example, the amino acid compounds may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxyl-apatite adsorption chromatography and hydrophobic interaction chromatography. In addition, the amino acid compound can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for the full length nascent gene product, or polypeptide fragments of the gene product.

Therefore, also provided are methods to construct the recombinant plants described herein, comprising: transforming a plurality of plant cells with a vector comprising an isolated nucleic acid compound which encodes SEQ ID NO 5; and causing at least some of the plant cells to grow into at least one plant; and selecting those plants which contain nucleic acid which encodes SEQ ID NO 5.

Moreover, there are provided methods to confer inducible resistance properties to a plant, comprising: transforming the plant with a vector comprising the nucleic acid sequences described herein. Since disease resistance is one characteristic conferred to a plant by the avirulence genes disclosed herein, an ideal method would be to activate the sequences disclosed herein for the amount of time and in quantities
necessary to fight infection, and no longer, since the genes cause hypersensitive reaction.

Also provided are methods to cause a hypersensitive response in a plant transformed with the nucleic acids described herein, comprising: introducing to the plant an agent or environmental conditions capable of inducing the inducible promoter of the vector, and providing the time and conditions needed to cause inducement, and causing inducement. Preferred agents are pathogens, and preferred environmental conditions are wounds. However, it is also within the scope of the present invention to select a plant part into which a vector is transformed, and selectively cause a hypersensitive response in response to a developmental stage of the plant, such as upon flowering or at a particular fruit developmental stage. For instance, it is desirable to cause damage in the abscission zone of a flower or fruit at a particular time, so as to either avoid the use of plant resources for flowering, or for ease in harvesting fruit.

Also provided are methods to cause death in plant tissue, wherein said plant tissue has been transformed with a vector of comprising an inducible promoter operatively-linked to a nucleic acid which encodes SEQ ID NO 5, comprising: causing expression of the nucleic acid which encodes SEQ ID NO 5 in the plant tissue so as to produce an expression product, and allowing the expression product adequate time and conditions to cause plant tissue death. This aspect of the invention could be used in herbicide development or for causing death of the plant prior to harvest of the fruit. For instance, it is desirable in many root and tuber crops to kill the leafy part of the plant prior to harvesting the root or tuber. The present invention can be utilized to further this goal, by selectively inducing the avirulence gene in the presence of the resistance gene product in, ie. the leafy part of the plant, at the appropriate developmental time, either by external or internal stimulus.

Lastly, there is provided a maize pathogen resistance gene, said gene being characterized as being approximately 1 centimorgan from
the restriction length polymorphism probe umc85 on the short arm of chromosome 6 of maize. A gene as above, which is further characterized as a dominant gene which controls a rapid necrotic reaction to the rice pathogen avirulence gene product encoded by *avrRx01* is preferred.

Transformation of plants with these sequences would be according to known procedures as described above. Plants can be grown according to known procedures.

**Examples** Identification and Cloning of the *avrRx01* Gene from *Xanthomonas oryzae pv. oryzicola*

**Example 1:** Construction of *X. oryzae pv. oryzicola* genomic library.

A library of *X. oryzae pv. oryzicola* strain BLS256 genomic DNA was constructed in the broad-host-range cloning vector pUFR034 (De Feyter et al., *88 Gene* 65 (1990)). Genomic DNA from *X. oryzae pv. oryzicola* was isolated by the lysozyme-sodium dodecyl sulfate (SDS) lysis procedure (Owen and Borman, *15 Nucleic Acids Res* 3631 (1987). The genomic DNA was partially digested with *Sau3A* and the fragments were treated with calf intestinal alkaline phosphatase according to Maniatis et al., *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y. 1982). The fragments were ligated to the broad host range cosmid vector pUFR034 (Kmr), (De Feyter et al., *88 Gene* 65 (1990)) which had been digested with *BamHI*. The ligated fragments were packaged into lambda phage in vitro by use of Gigapack II packaging extract (Stratagene, La Jolla, CA). Using these conditions, recombinant plasmids of 47 to 51 kb in length are selectively packaged. After transduction into *E. coli* DH5 MCR, white colonies were selected on media plates containing X-gal, IPTG and kanamycin (50 Fg/ml). About 97% of the colonies contained recombinant plasmids. One thousand five hundred colonies were randomly picked for the library. These were stored in microtiter plates in 30% glycerol at -80C (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y. 1982). Plasmid
DNA from randomly chosen clones was isolated, digested with EcoRI and HindIII, and separated in agarose gels. The insert size was about 30 - 40 kb and the fragmentation pattern was random.

**Example 2:** Identification of cosmid clone containing avrRxo1.

The cosmid library was screened for clones harboring the avirulence gene. Clones were mobilized into PXO99A, an azacytidine mutant strain of a closely related pathogen X. oryzae pv. oryzae (described in Hopkins et al., 5 Mol Plant-Microbe Interact 451 (1992), which does not induce the hypersensitive response (HR, a resistance response) on the maize line B73. Plasmid DNA from the clones was introduced to PXO99A by triparental mating as modified from Kelemu and Leach, 3 Mol Plant-Microbe Interact 59 (1990). Briefly, the library clones in E. coli DH5 MCR (donor) were grown overnight at 37°C on Luria agar (LA) with kanamycin (50 Fg/ml) and then transferred to the wells of microtiter plates each containing 80 Fl nutrient broth using a flame sterilized multiprong inoculator. The microtiter plates were incubated at 37°C with shaking for 4-6 hr. Eighty Fl of a 4-6-hr nutrient broth culture of the strain E. coli HB101 which contains the helper plasmid pRK2013 was added to each of the wells containing the library clones. The donor and helper were allowed to mate for 1 hr and then replica plated onto a lawn of PXO99A (recipient) on the surface of nutrient agar plates containing kanamycin (50 Fg/ml) and cephalexin (20 Fg/ml; used to inhibit E. coli). The plates were air dried and incubated at 30°C until transconjugants appeared. The transconjugants were picked and purified by streaking on nutrient agar containing kanamycin (50 Fg/ml) and cephalexin (20 Fg/ml). Seeds of the maize line B73 were grown in the greenhouse. The second and third leaves of seedlings (12 days after planting) were inoculated with bacterial suspensions of the transconjugants (prepared by suspending a loopful of bacterial cells from a peptone sucrose agar plate in 1 ml distilled water) by infiltrating bacteria into the intercellular spaces with a needleless syringe according to Reimers and Leach, 38 Physiol Mol Plant Pathol 39 (1991). Plants were examined for the induction of the HR 2 and 3 days
after infiltration. One transconjugant (V7) incited a strong HR on B73; this transconjugant contains the putative *avrRxo1* gene.

**Example 3: Subcloning of clone containing the putative *avrRxo1* gene**

Since many avirulence genes from the genus *Xanthomonas* are members of the *avrBs3* gene family, including *avrXa10* from *X. oryzae pv. oryzae*, DNA from clone V7 was analyzed from the presence of DNA homologous to this gene family. Plasmid DNA from clone V7 was digested with *Bam*HI and the resulting fragments were separated by electrophoresis on a 0.7% w/v agarose gel. DNA was transferred to Magna NT nylon transfer membrane (Micron Separations, Inc., Westboro, MA) and hybridized with a 3.1 kb *Bam*HI fragment of *avrXa10*, an avirulence gene from *X. oryzae pv. oryzae* (Hopkins et al., 5 *Mol Plant-Microbe Interact* 451 (1992)). The *avrXa10* probe was labelled with the enzyme horseradish peroxidase following the ECL direct nucleic acid labelling procedure (Amersham International, Buckinghamshire, England). Southern hybridization was performed as described in Leach et al., 3 *Mol Plant-Microbe Interact* 238 (1990). Hybridized bands were detected as specified in the ECL direct nucleic acid labelling and detection handbook (Amersham Life Sciences, Inc, Cleveland, OH). One *Bam*HI fragment (ca. 3.5 kb) hybridized strongly to *avrXa10*.

To determine if the *Bam*HI fragment that hybridized to *avrXa10* contained sequences important to avirulence, the fragment was extracted from the agarose gel using the Qiaex II gel extraction kit (Qiagen Inc., Valencia, CA) and ligated to the high copy number plasmid vector pUC19 (2.7 kb; Cbr) previously treated with calf intestinal alkaline phosphatase (Maniatis et al. 1982). The ligated product was transformed into *E. coli* DH5a. White colonies that developed on LA containing X-gal, IPTG and carbenicillin (100 ug/ml) were selected. Plasmid DNA from one pUC19 subclone was digested with *SphI* and the fragment containing the repeat domain was extracted from the agarose gel and ligated into pCH43 (4.6 kb; Cbr) treated with shrimp alkaline
phosphatase (Amersham Life Sciences, Inc., Cleveland, OH). The vector pCH43 is a high copy plasmid (based on pBluescript) modified so that the BamHI fragment (which contains the repeat domain from members of the avrXa10 gene family (Leach and White, 34 Annu Rev Phytopathol 153 (1996) can be cloned in frame into avrXa10 sequences for test of function and specificity (see Figure 1). The ligated insert and pCH43 vector fragments were transformed into E. coli DH5a and colonies were selected for resistance to carbenicillin (100 ug/ml). The orientation of insertion was determined by digesting plasmid DNA from selected subclones with PstI and EcoRI. Subclones that carried the insert in the both orientations were chosen. Plasmid DNA from these subclones (vector plus insert; ca. 8.1 kb) were digested with HindIII and ligated into pHM1 (13.3 kb; Spr), a broad host range cloning vector that is stably maintained in X. oryzae pv. oryzae. After transformation into E. coli DH5a, carbenicillin and spectinomycin resistant colonies were selected. Plasmid DNA was isolated from the pHM1 subclones and mobilized into PXO99A by electrotransformation (Choi and Leach, Mol Gen Genet, 1994, 244:383-390).

The electrotransformants were tested on B73 to determine their ability to induce the HR on this line. HR was induced on B73 by these subclones caused a slower (necrosis occurred 48-72 h after inoculation) response than that elicited by the parent strain from which the gene was cloned (X. o. pv. oryzicola BLS 256) and than that observed from the original cosmid clone V7. However, the response was an HR, suggesting that the 3.5 kb BamHI fragment containing the repeat domain was part of the functional avrRxo1 gene. Since the 3.5 kb BamHI fragment does not contain the entire coding region of the avrRxo1 gene, the cosmid clone V7 was subjected to deletion mutagenesis to obtain the entire avrRxo1 gene in one clone. Plasmid DNA from V7 was partially digested with Sau3A1. The resulting DNA fragments within the size range of 4 – 20 kb were excised from the gel and extracted using the gene clean kit. The purified DNA fragments were ligated to the broad host range cosmid vector pHMI (13.3 kb; Sp’) that was previously digested with BamHI and treated with calf intestinal
alkaline phosphatase. The ligated insert and vector fragments were transformed into *E. coli* DH5a and spectinomycin resistant transformants were selected. Plasmid DNA from the transformants were introduced into *X. oryzae* pv. *oryzae* strain PXO99A by triparental mating using *E. coli* pRK2013 as helper strain. Spectinomycin resistant transconjugants were selected and screened for the ability to induce the HR on B73.

A number of transconjugants induced the HR on B73. Three types of reactions were observed - 1) necrosis confined to point of inoculation, 2) patchy necrosis, and 3) necrosis of the entire infiltrated area, which required a longer time (48 to 72 hours after inoculation) for the onset of the HR. The clones which demonstrated such results contained inserts ranging from 3 to 6 kb. Some of the active clones had *BamHI* fragments that hybridized strongly to the 3.5 kb *BamHI* fragment of *avrRxo1* that is homologous to *avrXa10*. Some of the active clones do not have sequences homologous to the 3.5 kb homolog of *avrXa10*. These results indicate that the *avrXa10* homolog in clone V7 confers *avrRxo1* activity, but that suggests other avirulence genes are also present in cosmid clone V7. The subclones which (1) cause necrosis over the entire infiltrated area within 48-72 h, and (2) contain small insert fragments (less than 6 kb) which includes the 3.5 kb *BamHI* fragment were selected for further analysis and mapping.

**Example 4:** Sequence analysis of the *avrRxo1* gene, including repeat domain.

Plasmid DNA from the subclone harboring the 3.5 kb *BamHI* fragment (homologous to the *avrXa10* and cloned into pUC19) was linearized with *SmaI*. The linearized DNA was partially digested with *MscI* to create deletions (each of the repeats in the repeat domain bears an *MscI* restriction site). The *MscI* partial digests were re-circularized with T4 DNA ligase and transformed into *E. coli* DH5a. Plasmid DNA from deletion clones were digested with *KpnI* and *HindIII* to determine sizes of insert DNA. The plasmid DNA from seven deletion clones bearing
inserts within the size range of 0.9 to 2.5 kb were sequenced using
universal M13 forward sequencing primer.

The 3.5 kb BamHI fragment contains most of the coding region, but
lacks sequences for part of the carboxy terminus of the protein. These
sequences were obtained by sequencing an avirulence-active subclone
AC₆ (insert size approximately 22.4 kb) by using primers designed from
the sequence of the 3.5 kb region and directed outward.

The sequences were then obtained from deletion clones and the subclone
using an Applied Biosystems Model 377 PRISM automated sequencer
(Iowa State University DNA Sequencing Facility, Ames, IA 50011-3260).
DNA sequence analysis and translation to amino acid sequence were
carried out using the various utilities contained within BCM Launcher
(http://dot.imgen.bcm.tmc.edu:9331/seq-util/seq-util.html).

The avrRx01 gene is 3,720 bp in length and codes for a 1240 aa protein.
The gene contains 19.5 copies of a 102 bp directly repeated sequence
(translating to 34 aa each). As for other members of the avrBs3 gene
family, the repeats vary in a domain at aa positions 12 and 13. The
sequence and order of the repeats found in avrRx01 are shown (SEQ ID
No 3).

Although the present invention has been fully described herein, it
is to be noted that various changes and modifications are apparent to
those skilled in the art. Such changes and modifications are to be
understood as included within the scope of the present invention as
defined by the appended claims.

**Brief Summary of the Sequence Listing**

<table>
<thead>
<tr>
<th>SEQ ID NO</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA sequence of avrRx0</td>
</tr>
<tr>
<td>2</td>
<td>complementary strand of SEQ 1</td>
</tr>
<tr>
<td>3</td>
<td>DNA sequence of repeat domain of avrRx0</td>
</tr>
<tr>
<td>4</td>
<td>complementary strand of SEQ 3</td>
</tr>
<tr>
<td>5</td>
<td>amino acid sequence of avrRx0</td>
</tr>
<tr>
<td>6</td>
<td>amino acid sequence of repeat domain of avrRx0</td>
</tr>
</tbody>
</table>
WE CLAIM:

1. An isolated nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

   (a) a nucleic acid sequence which has at least 95% identity to a nucleic acid which is selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; wherein said identity can be determined using the DNAsis computer program and default parameters;

   (b) a nucleic acid sequence which encodes an amino acid sequence which has at least 95% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters;

   (c) a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; and

   (d) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); and a nucleic acid sequence of (c).
2. An isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1; and SEQ ID NO 3;

(b) a nucleic acid sequence which encodes an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6;

(c) a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; and

(d) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); and a nucleic acid sequence of (c).

3. An isolated nucleic acid molecule, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid comprising at least 2000 contiguous nucleotides of SEQ ID NO 1;

(b) a nucleic acid comprising at least 1000 contiguous nucleotides of SEQ ID NO 3;

(c) a nucleic acid which encodes an amino acid comprising at least 900 contiguous residues of SEQ ID NO 5;

(d) a nucleic acid which encodes an amino acid comprising at least 450 contiguous residues of SEQ ID NO 6;

(e) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); a nucleic acid sequence of (c); and a nucleic acid sequence of (d).
4. An isolated nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid comprising at least 2500 contiguous nucleotides of SEQ ID NO 1;

(b) a nucleic acid comprising at least 1500 contiguous nucleotides of SEQ ID NO 3;

(c) a nucleic acid which encodes an amino acid comprising at least 1250 contiguous residues of SEQ ID NO 5;

(d) a nucleic acid which encodes an amino acid comprising at least 750 contiguous residues of SEQ ID NO 6;

(e) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence of (a); a nucleic acid sequence of (b); a nucleic acid sequence of (c); and a nucleic acid sequence of (d).

5. A vector comprising a nucleic acid molecule of claim 1.

6. A vector comprising a nucleic acid molecule of claim 3.

7. A vector of claim 5, wherein said vector further comprises an inducible promoter operably linked to said nucleic acid compound.

8. A vector of claim 7, wherein said inducible promoter is tightly regulated.

9. A cell comprising at least one nucleic acid molecule of claims 1.
10. An isolated avrRxo amino acid compound, wherein said amino acid compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid sequence which has at least 95% identity to a nucleic acid which is selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; wherein said identity can be determined using the DNAsis computer program and default parameters;

(b) an amino acid sequence which has at least 95% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters; and

(c) an amino acid sequence which is encoded by a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3.

11. An isolated avrRxo amino acid compound of claim 10, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO 1; and SEQ ID NO 3; and

(b) an amino acid sequence selected from the group consisting of: SEQ ID NO 5; and SEQ ID NO 6, wherein said identity can be determined using the DNAsis computer program and default parameters;
12. An isolated avrRxo amino acid compound, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid comprising at least 2000 contiguous nucleotides of SEQ ID NO 1;

(b) an amino acid sequence which is encoded by a nucleic acid comprising at least 1000 contiguous nucleotides of SEQ ID NO 3;

(c) an amino acid comprising at least 900 contiguous residues of SEQ ID NO 5; and

(d) a nucleic acid which encodes an amino acid comprising at least 450 contiguous residues of SEQ ID NO 6;

13. An isolated avrRxo amino acid compound of claim 12, wherein said compound comprises an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence which is encoded by a nucleic acid comprising at least 2500 contiguous nucleotides of SEQ ID NO 1;

(b) an amino acid sequence which is encoded by a nucleic acid comprising at least 1500 contiguous nucleotides of SEQ ID NO 3;

(c) an amino acid comprising at least 1250 contiguous residues of SEQ ID NO 5; and

(d) an amino acid comprising at least 750 contiguous residues of SEQ ID NO 6.

14. A recombinant plant cell transformed with a vector of claim 5.

15. A recombinant seed comprising a vector of claim 5.

16. A recombinant plant embryo comprising a vector of claim 5.

17. A recombinant plant comprising a vector of claim 5.
18. A recombinant plant of claim 17, wherein said plant is a monocotyledon.

19. A recombinant plant of claim 17, wherein said plant is selected from the group consisting of: sorghum; soybean; sunflower; canola; alfalfa; rice; corn; wheat; cotton and barley.

20. A method to confer inducible resistance properties to a plant, comprising: transforming said plant with a vector of claim 8.

21. A method to cause a hypersensitive response in a plant transformed with a vector of claim 8, comprising: introducing to the plant an agent or environmental conditions capable of inducing the inducible promoter of the vector, and providing the time and conditions needed to cause inducement, and causing inducement.

22. A method to cause death in plant tissue, wherein said plant tissue has been transformed with a vector comprising an inducible promoter operatively-linked to a nucleic acid which encodes SEQ ID NO 5, comprising: causing expression of the nucleic acid which encodes SEQ ID NO 5 in the plant tissue so as to produce an expression product, and allowing the expression product adequate time and conditions to cause plant tissue death.

23. A maize pathogen resistance gene, said gene being characterized as being approximately 1 centimorgan from the restriction length polymorphism probe umc85 on the short arm of chromosome 6 of maize.

24. The gene of claim 24, which is further characterized as a dominant gene which controls a rapid necrotic reaction to the rice pathogen avirulence gene product, which gene product is characterized by SEQ ID NO 5.
<110> Leach, Jan E.
         Hulbert, Scot H.
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Val Ser Arg Thr Arg Leu Pro Ser Pro Pro Ala Pro Ser Pro Ala Phe
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Ser Ala Gly Ser Phe Ser Asp Leu Leu Arg Pro Phe Asp Pro Ser Ile
65 70 75 80

Pro Asp Thr Ser Leu Val Asp Ser Met Pro Ala Val Gly Thr Pro His
85 90 95

Thr Ala Ala Ala Pro Ala Glu Trp Asp Glu Met Gln Ser Ala Leu Arg
100 105 110

Ala Ala Asp Asp Pro Pro Pro Thr Val Arg Val Ala Val Thr Ala Ala
115 120 125

Arg Pro Pro Arg Ala Lys Pro Ala Pro Arg Arg Arg Ala Ala Gln Pro
130 135 140

Ser Asp Ala Leu Pro Ala Ala Gln Val Asp Leu Arg Thr Leu Gly Tyr
145 150 155 160

Ser Gln Gln Gln Glu Lys Ile Lys Pro Lys Gly Arg Ser Thr Val
165 170 175

Ala Gln His His Glu Ala Leu Val Gly His Gly Phe Thr His Ala His
180 185 190

Ile Val Ala Leu Ser Gln His Pro Ala Ala Leu Gly Thr Val Ala Val
195 200 205

Thr Tyr Gln His Ile Ile Thr Ala Leu Pro Glu Ala Thr His Glu Asp

SUBSTITUTE SHEET (RULE 26)
Ile Val Gly Val Gly Lys Gln Trp Ser Gly Ala Arg Ala Leu Glu Ala
Leu Leu Thr Asp Ala Gly Glu Leu Arg Gly Pro Pro Leu Gln Leu Asp
Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg Gly Gly Val Thr Ala Val
Glu Ala Val His Ala Ser Arg Asn Ala Leu Thr Gly Ala Pro Leu Asn
Leu Thr Pro Ala Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp
His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn Gly Gly
Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
Gln Asp His Asp Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser His
Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala
Ser Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala
Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val
Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp
465 470 475 480

Gln Val Val Ala Val Ala Gly Asn Ile Gly Gly Lys Gln Ala Leu Glu
485 490 495

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr
500 505 510

Pro Asp Gln Val Val Ala Ile Ala Asn Asn His Gly Gly Lys Gln Ala
515 520 525

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly
530 535 540

Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser His Gly Gly Lys Ser
545 550 555 560

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
565 570 575

His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn Ile Gly
580 585 590

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
595 600 605

Gln Ala His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Asn Asn
610 615 620

Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
625 630 635 640

Leu Cys Gln Glu His Gly Leu Thr Leu Asp Gln Val Val Ser Ile Ala
645 650 655

Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
660 665 670

Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala
675 680 685

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
690 695 700

Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val
705 710 715 720

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Val Ala Ile Ala Asn Asn Ser Gly Gly Lys Gln Ala Leu Glu Thr Val
725 730 735
Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Leu Ala
740 745 750
Gln Val Val Ala Ile Ala Ser Asn Gly Gly Lys Gln Ala Leu Glu
755 760 765
Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr
770 775 780
Pro Ala Gln Val Val Ala Ile Ala Asn Asn Gly Gly Lys Gln Ala
785 790 795 800
Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
810 815
Leu Thr Pro Ala Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
820 825 830
Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp
835 840 845
His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn Gly Gly
850 855 860
Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
865 870 875 880
Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn
885 890 895
Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
900 905 910
Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala
915 920 925
Ser Asn Gly Gly Lys Gln Ala Leu Glu Ser Ile Val Ala Gln Leu Ser
930 935 940
Arg Pro Tyr Pro Ala Leu Thr Ala Leu Thr Asn Asp His Leu Val Ala
945 950 955 960
Leu Ala Cys Leu Gly Gly Arg Pro Ala Leu Asp Ala Val Lys Lys Gly
970 975 980

SUBSTITUTE SHEET (RULE 26)
Leu Pro His Ala Pro Glu Leu Ile Arg Arg Ile Asn Arg Arg Ile Pro

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

Leu Glu Phe Phe Gln Cys His Ser His Pro Ala Gln Ala Phe Asp Asp

Ala Met Thr Gln Phe Gly Met Ser Arg Gln Gly Leu Val Gln Leu Phe

Arg Arg Val Gly Val Thr Glu Phe Glu Ala Arg Tyr Gly Thr Leu Pro

Pro Ala Ser Gln Arg Trp Asp Arg Ile Leu Gln Ala Ser Gly Met Lys

Arg Ala Lys Pro Ser Cys Ala Ser Ala Gln Thr Pro Asp Gln Ala Ser

Leu His Gly Phe Ala Asp Ser Leu Glu Arg Asp Leu Asp Ala Pro Ser

Pro Met His Gln Gly Gln Thr Arg Ala Ser Ser Arg Lys Arg Ser

Arg Ser Asp Arg Ala Val Thr Gly Pro Ser Ala Gln Gln Ala Val Glu

Val Arg Val Pro Gln Arg Asp Ala Leu His Leu Pro Leu Ser Trp

Ser Val Lys Arg Pro Arg Thr Arg Ile Gly Gly Gly Leu Pro Asp Pro

Gly Thr Pro Ile Ala Ala Asp Leu Ala Ala Ser Ser Thr Val Met Trp

Glu Gln Asp Ala Pro Phe Ala Gly Ala Ala Asp Asp Phe Pro Ala

Phe Asn Glu Glu Leu Ala Trp Leu Met Glu Leu Leu Pro Gln Ser

Gly Ser Val Gly Gly Thr Ile
Xanthomonas oryzae

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino Acid Sequence</th>
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<tbody>
<tr>
<td>1-5</td>
<td>Leu Thr Pro Ala Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly</td>
</tr>
<tr>
<td>6-10</td>
<td>Lys</td>
</tr>
<tr>
<td>11-15</td>
<td>Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp</td>
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<tr>
<td>16-20</td>
<td>30</td>
</tr>
<tr>
<td>21-25</td>
<td>His Gly Leu Thr Pro Asp Gln Val Ala Ile Ala Ser Asn Gly Gly</td>
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<tr>
<td>26-30</td>
<td>45</td>
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<tr>
<td>31-35</td>
<td>Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys</td>
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<tr>
<td>36-40</td>
<td>60</td>
</tr>
<tr>
<td>41-45</td>
<td>Gln Asp His Asp Leu Thr Pro Asp Gln Val Ala Ile Ala Ser His</td>
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<tr>
<td>46-50</td>
<td>80</td>
</tr>
<tr>
<td>51-55</td>
<td>Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val</td>
</tr>
<tr>
<td>56-60</td>
<td>95</td>
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<tr>
<td>61-65</td>
<td>Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Ala Ile Ala</td>
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<tr>
<td>66-70</td>
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<tr>
<td>71-75</td>
<td>Ser Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu</td>
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<tr>
<td>76-80</td>
<td>125</td>
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<tr>
<td>81-85</td>
<td>Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala</td>
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<td>86-90</td>
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<tr>
<td>91-95</td>
<td>Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg</td>
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<tr>
<td>101-105</td>
<td>Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val</td>
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<tr>
<td>111-115</td>
<td>Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val</td>
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<tr>
<td>116-120</td>
<td>190</td>
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<tr>
<td>121-125</td>
<td>Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp</td>
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</tbody>
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11/13
Gln Val Val Ala Val Ala Gly Asn Ile Gly Gly Lys Gln Ala Leu Glu
210       215       220
Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr
225       230       235       240
Pro Asp Gln Val Val Ala Ile Ala Asn Asn His Gly Gly Lys Gln Ala
245       250       255
Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly
260       265       270
Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser His Gly Gly Gly Lys
275       280       285
Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
290       295       300
His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn Ile Gly
305       310       315       320
Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
325       330       335
Gln Ala His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Asn Asn
340       345       350
Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
355       360       365
Leu Cys Gln Glu His Gly Leu Thr Leu Asp Gln Val Val Val Ser Ile Ala
370       375       380
Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
385       390       395       400
Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala
405       410       415
Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
420       425       430
Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val
435       440       445
Val Ala Ile Ala Asn Asn Ser Gly Gly Lys Gln Ala Leu Glu Thr Val
450       455       460
Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr Leu Ala
465 470 475 480
Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu
485 490 495
Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp His Gly Leu Thr
500 505 510
Pro Ala Gln Val Val Ala Ile Ala Asn Asn Asn Gly Gly Lys Gln Ala
515 520 525
Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
530 535 540
Leu Thr Pro Ala Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
545 550 555 560
Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Asp
565 570 575
His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn Gly Gly
580 585 590
Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
595 600 605
Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala Ser Asn
610 615 620
Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
625 630 635 640
Leu Cys Gln Asp His Gly Leu Thr Pro Asp Gln Val Val Ala Ile Ala
645 650 655
Ser Asn Gly Gly
660