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(54) Title: *ARABIDOPSIS THALIANA* CYCLIC NUCLEOTIDE-GATED ION CHANNEL/*DND* GENES; REGULATORS OF PLANT DISEASE RESISTANCE AND CELL DEATH

(57) Abstract: The cell death response known as the hypersensitive response (HR) is a central feature of gene-for-gene plant disease resistance. Plants also defend against pathogens via multigenically controlled broad-spectrum defense responses, such as those modulated by salicylic acid. The *DND* (Defense, No Death) loci of *Arabidopsis thaliana* regulate the extent of broad-spectrum disease resistance against a broad range of viral, bacterial, oomycete and fungal pathogens. Plants lacking a functional copy of the *DND1* or *2* gene are defective in HR cell death but exhibit successful gene-for-gene disease resistance. Plants lacking a functional copy of the *DND1* or *2* gene also exhibit an enhanced broad-spectrum disease resistance phenotype. The *DND1* and *2* gene products are identical to previously known cDNAs termed *AtCNGC2* and *1*, respectively, that encode apparent cyclic nucleotide-gated ion channel proteins. The identification of the *CNGC/DND* genes as regulators of disease resistance and host cell death, and the availability of *CNGC/DND* gene sequence information, provide new possibilities for controlling a wide variety of plant diseases.

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ARABIDOPSIS THALIANA CYCLIC NUCLEOTIDE-GATED ION CHANNEL/*DND* GENES; REGULATORS OF PLANT DISEASE RESISTANCE AND CELL DEATH

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority from United States Provisional Patent Application No. 60/145,310, filed July 23, 1999.

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BACKGROUND OF THE INVENTION

This invention relates to plant physiology, in particular, plant genes, termed cyclic nucleotide-gated ion channel genes or *DND* (Defense, No Death) genes as regulators for plant diseases and methods for controlling plant diseases.

Numerous plant diseases have plagued humankind from the dawn of time. They have caused major economic disruptions, substantial crop losses to individual growers, famines and even disruption of entire cultures. Plant diseases are estimated to cause in excess of nine billion US dollars in pre-harvest loss of cultivated crop plants each year.

Growers presently control plant diseases with a combination of germplasm choice (plant genetics), adaptive plant culture practices, and exogenous pesticidal treatments. All three strategies are widely in use. However, for a given crop and disease, control measures may only be partially effective, or not affordable, or may not be available at all. In some cases, growers entirely avoid cultivation of a valued plant species and shift to cultivation of other species because of disease problems.

Gene-for-gene resistance is a form of plant disease resistance that is exploited widely by plant breeders for crop plants [McIntosh, R.A., et al., (1995) *Wheat Rusts: An Atlas of Resistance Genes* Commonwealth Scientific and Industrial Research Organization, Australia, and Kluwer, Dordrecht, The Netherlands; Crute, I.R. et al., (1996) *Plant Cell* **8**:1747-1755; Agrios, G.N. (1997) *Plant Pathology* (Academic, San Diego); Bent, A.F. (1996) *Plant Cell* **8**:1757-1771]. The name "gene-for-gene" denotes the dependence of this resistance on matched specificity between a plant disease resistance gene and a pathogen avirulence gene [Flor, H.H. (1941) *Phytopathology* **32**:653-669]. In a process that is reminiscent of mammalian antibody-antigen interactions, these genes control receptor-ligand interactions that activate complex defense responses [Bent, (1996) supra; Alfano, J.R. et al. (1996) *Plant Cell* **8**:1683-1698; Hammond-Kosack, K.E. et al. (1996) *Plant Cell* **8**:1773-1791].

There are thousands of resistance genes that mediate the recognition of specific fungal, bacterial, viral, or nematode pathogen strains. The strong defense response that is triggered after a gene-for-gene interaction includes synthesis of antimicrobial enzymes and metabolites, generation of signaling molecules that activate defense in neighboring cells and reinforcement of plant cell walls surrounding the site of infection [Bent, (1996) supra; Hammond-Kosack, (1996) supra; Dangl, J.L. et al. (1996) *Plant Cell* **8**:1793-1807]. One of the most prominent features of gene-for-gene defense is the death of infected plant cells within hours after initial contact with pathogen, a process known as the hypersensitive response (HR) [Stakman, E.C., (1915) *J. Agric. Resd.* **4**:193-199; Goodman, R.N. et al. (1994) *The Hypersensitive Reaction in Plants to Pathogens: A Resistance Phenomenon* (Am. Phytopathol. Soc., St. Paul)]. HR cell death is a programmed cell death response that bears features of the apoptotic cell death processes that occur in other metazoan organisms [Dangl, (1996) supra]. Although HR cell death is a hallmark of gene-for-gene disease resistance, the relative importance of cell death in this form of disease resistance is not clear and may vary depending on the target pathogen species [Hammond-Kosack, (1996) supra; Dangl, (1996) supra; Stakman, (1915) supra; Goodman, (1994) supra].

Multiple plant defense responses are activated in response to pathogen infection [Bent, A.F. et al., (1999) *Advances in Agronomy* **66**:251-298; Dixon, R.A. et al. (1990) *Adv. Genet.* **28**:165-234; Ryals, J.L. et al. (1996) *Plant Cell* **8**:1809-1819; Hammond-Kosack (1996) supra]. While gene-for-gene systems control early and strong activation of plant defenses following recognition of the invading pathogen, many of the same plant defenses are activated more gradually or to a lesser extent in other forms of disease resistance. Even disease-susceptible plants activate a wide variety of defenses, appreciably slowing disease progression [Delaney, T.P. et al. (1994) *Science* **266**:1247-1250; Glazebrook, J. et al. (1996) *Genetics* **143**:973-982]. Although some defense responses are particularly effective against specific pathogens, many plant species induce multifactorial defenses that are somewhat generic. These general or "broad-Spectrum" defense responses are often effective against a wide variety of viral, bacterial and fungal pathogens [Bent, (1999) supra; Dixon, (1999) supra; Ryals, (1996) supra]. Salicylic acid has been shown to be a key endogenous mediator that promotes expression of a diverse set of plant defenses [Delaney, (1996) supra; Gaffney, T. et al. (1993) *Science* **261**:754-756]. Salicylic acid can be required for effective gene-for-gene resistance, for other localized defense responses, and for systemic acquired resistance (SAR) [Ryals, (1996) supra]. Other defense pathways have been identified that are apparently independent of salicylic acid, such as many jasmonic acid-dependant defense responses [Penninckx, I.A. et al. (1996) *Plant Cell* **8**:1809-1819]. Multigenically controlled defense pathways form important barriers to infection, and plant breeding efforts are often devoted to improvement of these "quantitative" types of resistance [Agrios, (1997) supra].

Yu et al. [Yu et al. (1998) *Proc. Natl. Acad. Sci. USA* **95**:7819-7824; Yu et al. (2000) *Mol. Plant-Microbe Interactions* 13:277-286] identified two *Arabidopsis* mutants, *dnd1 and dnd2*, that do not develop the HR response to avirulent *P. syringae* pathogens. These *dnd* mutants exhibited gene-for-gene restriction of pathogen growth in the absence of extensive HR cell death and also exhibited a constitutive systemic acquired resistance phenotype. This constitutive induction of systemic acquired resistance may substitute for HR cell death in potentiating the stronger gene-for-gene defense response.

Advances in molecular biology and genetic engineering now make it feasible to tailor important crops to better cope with pathogens and reduced losses to plant diseases. Many of the major crop species can routinely be transformed and regenerated [Christou, (1996) *Trends Plant Sci.* **1**:423-431]. However, this type of genetic engineering requires a knowledge of the molecular processes involved.

In order to provide a basis for developing more efficient means to control diseases in plants, the present invention describes a class of plant genes exemplified by two *Arabidopsis* genes termed *DND* (Defense, No Death) 1 and 2 which were discovered herein to encode proteins formerly identified in the literature as putative cyclic nucleotide-gated ion channels (cDNAs of *AtCNGC2* and *AtCNGC1*, respectively) [Kohler, C. et al (1999) *Plant J.* **18**:97-104; Kohler, C. et al. (1998); *Plant Physiol.* **116**:1604; Leng et al. (1999) *Plant Physiol* **121**:753-761]. Therefore, the terms "*DND1*" and "*DND2*" as used herein are intended to be synonymous with *AtCNGC2* and *AtCNGC1*, respectively. Note, however, that in this previous work by others on *AtCNGC2* and *AtCNGC1*, no association was made with plant disease resistance, cell death or related whole-plant phenotypes. The *AtCNGC/DND* genes regulate disease resistance, i.e. modified forms of these genes cause enhanced resistance against a broad range of viral, bacterial and fungal pathogens in the absence of cell death. Therefore, manipulation of these genes or other related genes allows the generation of plants with improved disease resistance.

SUMMARY OF THE INVENTION

The object of the invention is to provide methods for improving disease resistance in plants. A second object of the invention is to provide methods for control of cell death in plants. The plant genes *DND* (Defense, No Death) 1 and 2 of *Arabidopsis thaliana* described herein regulate broad-spectrum disease resistance and cell death in plants. The nucleotide coding sequences of the *DND* 1 and 2 genes of the present invention are identical to previously known cDNA molecules that encode proteins that function as cyclic nucleotide-gated ion channels 2 (*AtCNGC2*) and 1 (*AtCNGC1*), respectively. Such cyclic nucleotide-gated ion channels are ubiquitous in plants, generally. Plants that do not express *AtCNGC/DND* genes due to a mutation exhibit elevated resistance against a broad range of viral, bacterial, and fungal pathogens, and also exhibit a decrease in the HR cell death response to avirulent pathogens and a decrease in cell death induced by Fumonisin B1 toxin. Therefore, this invention discloses various methods for improving disease resistance by modifying the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene or gene product, or genes or gene products in other plants that share substantial structural or functional similarity to the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene or gene product. The modifying means include, but are not limited to transcriptional or translational down-regulation, mutations including nucleotide substitution, deletion or insertion, inactivation of the gene or gene product, and chemical inhibition of the gene product. These genes may also be down-regulated or inactivated by antisense technology, sense-strand suppression, virus-induced gene silencing, double strand RNA and other inactivation methods known in the art.

The invention further includes a transformed or genetically modified plant, plant tissue or seed made by the described method.

The invention discloses methods for identifying other *AtCNGC2/DND1* or *AtCNGC1/DND2* related disease resistance genes or structural or functional homologs thereof. The *AtCNGC2/DND1* or *AtCNGC1/DND2* related genes or homologs or gene products thereof thus identified can be modified as described herein to improve disease resistance. These genes and gene products can be used in a screen to identify inhibitors for enhancing disease resistance in plants.

The invention also provides use of genetic markers for improving plant disease resistance via prevailing plant breeding practices. The genetic markers are identified because of their similarity to or close genetic proximity to *AtCNGC2/DND1* or *AtCNGC1/DND2* or their proximity to homologs of *AtCNGC2/DND1* or *AtCNGC1/DND2*.

The identification of the *CNGC/DND* genes as disease resistance regulators provides additional means to identify other molecules which interact with them in exhibiting disease resistance. These include effector genes or proteins or chemicals which interact with a *CNGC/DND* gene or gene product or homolog.

The methods disclosed in the invention to improve disease resistance in plants may also be used to control disease-induced cell death. Accordingly, the invention includes: A method for controlling cell death in a plant by down-regulating, mutating or inactivating a *CNGC/DND* gene or gene product of the plant. Specifically, such method can include inactivating a *CNGC/DND* gene using an appropriate *CNGC/DND* antisense or sense DNA. Accordingly, the invention includes antisense DNA molecules of *DND1* or *DND 2* genes or similar genes from other plant species. The invention further includes a transformed or genetically modified plant, plant tissue or seed made by the described method, or transformed to express the described antisense or sense DNA.

In another aspect, the invention provides a method for improving pathogen resistance of a plant by down-regulating, mutating or inactivating a cyclic nucleotide-gated ion channel gene or gene product or a homolog thereof of the plant. Similarly, the invention provides transformed or genetically modified plant, plant tissue or seed made by the described method.

The invention further provides a method for identifying a disease-resistance gene by screening for a cyclic nucleotide-gated ion channel gene, including *AtCNGC2/DND1* and *AtCNGC1/DND2* genes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1D shows HR cell death defect in *dnd1* mutant. Leaves of wild-type parent (Col) and *dnd1* mutant (*dnd1*) plants were inoculated with a high dose (2 x 10^8 cfu/ml) of avirulent, HR-stimulating *P. syringae* pv. *glycinia* Rct 4 pV288 (Psg *avrRp2*$^+$) or the isogenic, nonavirulent control strain *P. syringae* pv. *glycinia* Race 4 pVSP61(Psg). At 24 h postinoculation, leaves were harvested, fixed, and examined for autofluorescent dead cells by using a fluorescence microscope. Fig. 1C shows the edge of an inoculated zone, revealing confluent cell death in response to bacteria only on the left (inoculated) side.

Figs. 2A-2B illustrate the growth of bacteria within plant leaves. Fig. 2A shows *Arabidopsis* lines Col (Col-0 wild-type, *RPS2/RPS2; DND1/DND1*), *rps2* (Col-0 *rps2-201/rps2-201; DND1/DND1*), and *dnd1* (Col-0 and *RPS2/RPS2; dnd1/dnd1*) inoculated with *P. syringae* pv. *tomato* DC3000 pV288 (*avrRpt2*$^{+}$). Fig. 2B shows *Arabidopsis* lines Col-0 and *dnd1* inoculated with isogenic *P. syringae* pv. *tomato* DC3000 differing by the presence (pAvrRpm1, filled symbols) or absence (pVSP61, open symbols) of avirulence gene *avrRpm1* carried on plasmid pVSP61. Both plant lines are *RRM1/RPM1* genotype. All data points are mean $\pm$ SD.

Figs. 3A-3C show pathogenesis-related gene expression monitored by RNA blot analysis of Col-0 wild-type (Col) and Col-0 *dnd1/dnd1* mutant (*dnd1*) plants. Fig. 3A illustrates β-glucanase expression 72 h after treatment of leaves with 10 mM $MgCl_2$ containing no pathogen (⌀), the nonavirulent control strain *P. syringae* pv. *tomato* DC3000 pVSP61 (vir), or the isogenic *avrRp2*-expressing strain *P. syringae* pv. *tomato* DC3000 pV288 (*avr*). Fig. 3B illustrates PR-1 expression 24 h after treatment as in Fig. 3A. Fig. 3C shows Phosphorimager quantification of PR-1 expression from blot shown in Fig. 3B, normalized to level of constitutive β-ATPast mRNA. Similar results were obtained in multiple experiments.

Figs. 4A-4B show the levels of salicylic acid and glucoside-conjugated salicylic acid compounds in Col-0 and mutant Col-0 *dnd1-1dnd1-1* or Col-0 *dnd2-1/dnd2-1* plants.

Figs. 5A and 5B illustrate that the *dnd1* and *dnd2* mutant plants show more resistance to cell death induced by Fumonisin B1 (an inhibitor of ceramide synthase) compared to the wild type *Arabidopsis*. Fig. 5A is a dose-response curve of Fumonisin B1 generated using control (Col) and *dnd1* mutant plants. Fig. 5B shows the delayed response of the *dnd2* mutant plants after Fumonisin treatment compared to the wild type *Arabidopsis*. The Y axis in both graphs indicates the severity of necrosis rated on a 0-5 scale (0=no lesions, 5=complete necrosis).

Fig. 6 shows nucleotide sequences of the genomic region containing *AtCNGC2/DND1* gene, 5,897 nucleotides in length. The notable features are as follows: nt 1632; 5' end of the *AtCNGC2/DND1* cDNA, nt 1663; ATG putative start codon, nt 1716; end of exon 1 of *AtCNGC2/DND1* gene, nt 2088-2763; exon 2, nt 2928-3143;exon 3, nt 3333-3652; exon 4, nt 3747-3863; exon 5, nt3953-4192; exon 6, nt 4275-4363; exon 7, nt 4478-5153; 3' end of *AtCNGC2/DND1* cDNA, nt 4987 and TAA putative stop codon. The *dnd1* mutant described in the invention contains G to A point mutation creating a stop codon at position 3101 nt as underlined. The sequences shown herein are identical to those of SEQ ID NO:1

Fig. 7 shows the amino acid sequence (SEQ ID NO:3) of the protein encoded by the *DND1* gene.

Fig. 8 shows the nucleotide sequence of *AtCNGC2/DND1* cDNA (SEQ ID NO:2).

Fig. 9 illustrates the results of the complementation studies. The three complementing cosmids derived from BAC3H2 (1A8, 1H2 and 1H3) are depicted by solid bars. Striped bars represent cosmids that failed to complement the *dnd1* dwarf phenotype. Numerical data are the number of size-complemented plants out of the total number of T2 plants examined for each cosmid.

Fig. 10 shows response of T2 Col-0 *dnd1/dnd1* plants transformed with cosmids 1A8 and 1H2. T2 plants segregated 3:1 (wild type:dwarf) for size. Plants of both types were inoculated with Psg R4 *avrRpt2* or with Psg R4 (no *avr*). HR was scored 24 hours post inoculation. The degree of HR was scaled from 0 (no HR) to 5 (severe HR) respectively. For plants of dwarf stature, 7, 3, and 4 plants were tested for *dnd1*, 1A8, and 1H2 respectively. The number indicates the average of three leaves per plant (*avr*) and one leaf per plant (no *avr*) for wild-type size plant. Dwarf plant scores represent the average of at least six inoculated leaves (*avr*) or three leaves (no *avr*).

Fig. 11 shows growth of virulent *P. syringae* pv. *tomato* (pst) DC3000 in Col-0 *dnd1/dnd1* plants transformed with cosmid 1H3. Six-week old T2 plants segregating 3:1 (wild-type:dwarf) size were inoculated with Pst DC3000 with no *avr* gene. Bacterial growth was sampled 0, 2, and 4 days post inoculation for T2 plants of wild-type size, as well as the Col-0 and *dnd1* controls. For T2 plants of dwarf stature, bacterial growth was sampled only at 3 days post inoculation (depicted by the X).

Fig. 12 shows complementing cosmids and subclones. Complementing cosmids are represented by solid bars. Complementing subclones are represented by spotted bars and are depicted immediately above their parent cosmid. Cosmids and subclones that failed to complement are represented by white bars. Subclones were generated using EcoRI and/or XbaI, except where noted.

Fig 13 shows the nucleotide sequence of the genomic region containing the *Arabidopsis DND2 (AtCNGC1)* gene (SEQ ID NO:4).

Fig. 14 shows the nucleotide sequence of *DND2 (AtCNGC1)* cDNA (SEQ ID NO:5).

Fig. 15 shows the amino acid sequence of the protein encoded by the *DND2(AtCNGC1)* gene (SEQ ID NO: 6).

Fig. 16 shows the results of the complementation studies of *dnd2* small rosette size phenotype by transformation with the *Arabidopsis* genomic DNA fragment shown in Fig. 13 (SEQ ID NO:4), encoding *AtCNGC1/DND2*. "Col + vector" represents the wild type plants transformed with vector only, "dnd2+vector" represents the *dnd2* mutant plants transformed with vector only, and "dnd2+AtCNGC1" represents the *dnd* mutant plants transformed with a vector containing the *Arabidopsis* genomic DNA frgment shown in Fig. 13 (SEQ ID NO:4), encodidng *AtCNGC1/DND2*.

DETAILED DESCRIPTION OF THE INVENTION

As used in the present invention, the following terms are defined as follows:

The term "down-regulation", as used herein, refers to a general method of reducing the level of gene products (RNA or protein). Thus, down-regulation of a gene may be achieved either transcriptionally or translationally. For example, an antisense molecule may be introduced into a cell or tissue to down-regulate the gene from which the antisense molecule is derived.

The term "mutation" as used herein refers to a modification of the natural nucleotide sequence of a nucleic acid molecule made by deleting, substituting, or adding a nucleotide(s) in such a way that the protein encoded by the modified nucleic acid is altered. The resulting proteins often exhibit altered functionality.

The term "antisense molecule" as used herein is intended to mean a single stranded nucleic acid molecule consisting of the complementary nucleotides of a sense molecule. The sense molecule in general refers to the strand of DNA or RNA which has the sequence of mRNA encoding the protein.

The term "disease resistance" or "pathogen resistance" as used herein refers to any process by which a plant response to pathogen attack functions to enhance the plant's ability to survive and/or maintain productivity despite that attack.

"Improved resistance" in a plant variety means that the damage associated with pathogen attack in that variety is reduced when compared to a control variety, as measured by an art-recognized criterion. The ultimate goal of improved resistance is to provide a higher crop yield, on average, from the variety having improved resistance, compared to the control. Since crop yields require time-consuming field trials, various laboratory tests have been devised to measure resistance in individual plants, such tests being art-recognized as predictive of improved yield in the presence of the pathogen. These tests include, but are not limited to, measurement of pathogen growth in the infected plant, measurements of extent of necrosis, plant cell death and hypersensitivity response. Such measurements are generally preferred because they can be conducted under controlled conditions, controlled pathogen level, timing of pathogen introduction, temperature, humidity and the like. In a specific example described herein, disease resistance is measured as restriction of pathogen growth, i.e., growth of an inoculated pathogen (i.e. *P. syringae pv. tomato*) was much less in the *dnd* mutant compared to the wild type. Thus, when a plant is modified to exhibit improved disease resistance, or improved pathogen resistance according to the methods described herein, it is understood that similar growth restriction to a given pathogen ultimately would result in reduced damage to the plant and higher crop yield.

The term "gene" refers to a deoxynucleic acid molecule that encodes a protein or peptide upon transcription and translation. Thus, "gene product" as used herein refers to either an RNA molecule or protein which is generated by expression of a given gene.

"*DND* gene" as used herein is intended to mean any gene that has structural homology to *DND1, DND2* or other genes whose product would closely resemble that of an intact or mutated cyclic nucleotide-gated ion channel gene and that, when down-regulated, mutated, or inactivated, causes improved resistance or improved cell death traits as described in the present invention. Accordingly, it includes not only the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene of *Arabidopsis* but also those corresponding or related genes of other plant species which have structural or functional homology with the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene disclosed herein. It will be understood in the art that variant structures of the *AtCNGC2/DND1* or *AtCNGC1/DND2* can exist in other plants, and that such variants can be identified, as herein described, by structural homology, by functional homology, or by similarity of phenotype in genetic analyses, or by any combination of the foregoing.

The meaning of a "homolog" as used in the present invention is intended to include any gene or gene product which has a structural or functional similarity to the gene or gene product in point. Accordingly, a structural homolog of the *CNGC/DND* gene is defined as one hybridizing with the *Arabidopsis AtCNGC2/DND1* (SEQ ID NO: 2) or *CNGC1/DND2* (SEQ

ID No: 5) genomic DNA or cDNA at a herein defined level of stringency of the conditions of hybridization, at low stringency, preferably at medium stringency or more preferably at high stringency. A second and equally valid definition of "homolog" is a gene for which the derived amino acid sequence of a translation product bears significant similarity to previously characterized cyclic nucleotide-gated ion channels, including the hallmark six transmembrane domains, a pore domain between the fifth and six transmembrane domain, and a cytoplasmic cyclic nucleotide interaction domain [Zagotta and Siegelbaum (1996) *Ann. Rev. Neurosci.* **19**:235-263; Kohler, et al. (1999) supra]. As a third and equally valid definition of "homolog," a functional homolog of a *DND* gene product is a cyclic nucleotide-gated ion channel protein that can potentially function or can be caused by mutation, down-regulation or chemical inhibition to function as a disease resistance regulator or a regulator of cell death. A functional homolog of the *CNGC/DND* gene product is one which potentially functions upon modification as a regulator of disease resistance and/or cell death.

The present invention discloses two plant genes, *DND1* and *DND2* of *Arabidopsis thaliana* as regulators of disease resistance and cell death. Plants homozygous for mutated *DND1 or DND2* gene exhibit enhanced disease resistance in the absence of cell death. Therefore, the manipulation of the *DND1 or DND2* gene offers new possibilities of controlling various plant diseases.

To address the relationship between HR cell death, resistance gene-mediated defense signal transduction, and the actual restriction of pathogen growth, mutants of *Arabidopsis thaliana* that were deficient in the HR were isolated and characterized. A mutagenized M_2 population of *Arabidopsis* line Col-0, which expresses the *RPS2* resistance gene, was screened by inoculating plants with a strain of the bacterial plant pathogen *P. syringae* pv. *glycinia* expressing the *RPS2*-complementary avirulence gene *avrRP2* [Kunkel, B.N., et al. (1993) *Plant Cell* **5**:865-875]. An extremely high titer of pathogen, 2 x 10^8 cfu/ml, was used so that plants undergoing a wild-type HR would exhibit visible collapse of leaf tissue.

Two of the mutants isolated from this screen were called *dnd1* and *dnd2*. These mutants exhibited several similar phenotypes; both are recessive to wild type, homozygous mutant plants show an extreme reduction in the extent of cell death in response to avirulent *P. syringae*, and dwarfism. Because *dnd1* and *dnd2* mutants were analyzed in a similar manner and found to exhibit similar mutant phenotypes, the following description is taken largely from the *dnd1* mutant analysis. However, it is easily understood by a person skilled in the art that these methods are readily applicable to the *dnd2* mutant analysis.

The *dnd1* mutant was recovered from this screen as a line displaying reduced rosette size and a clear HR$^-$ phenotype. Progeny lines derived from the *dnd1* mutant failed to produce an HR not only when inoculated with pathogens expressing *avrRpt2* but also in response to *P. syringae* that express avirulence genes *avrRpm1* or *avrB* (Kunkel, (1993) supra; Bisgrove, S.R. et al. (1994) *Plant Cell* **6**:927-933]. Two separate resistance genes (*RPS2* and *RPM1)* control responsiveness to these three separate avirulence genes. Accordingly, it is predicted that the *dnd1* line is disrupted in a common component of the plant defense response that is shared by initially distinct gene-for-gene signal transduction pathways.

To confirm the absence of hypersensitive cell death in response to avirulent pathogens in the *dnd1* mutant, fluorescence microscopy was used to monitor cells within inoculated leaf tissue [Klement, Z. et al. (1990) in *Methods in Phytobacteriology*, eds. Klement, Z., Rudolph, K. & Sands, D.C. (H. Stillman, Budapest), pp. 469-473]. Plant cells that undergo the HR display a marked increase in fluorescence due primarily to the production and release of phenolic compounds upon cell death. In "low titer" experiments, *P. syringae* pv. *glycinia* expressing *avrRP2* were introduced into leaf mesophyll tissue at a concentration of ≈5 x 10^5 cfu/ml, a dose at which a majority of the plant cells are not initially in contact with pathogen. As expected, leaves from the wild-type parental line infected at this dose with *P. syringae* expressing *avrRP2* contained numerous isolated autofluorescent cells. In contrast, very few autofluorescent foci were present in *dnd1* leaves inoculated with the same avirulent strain. The *dnd1* leaves instead resembled uninoculated leaves or leaves inoculated with the nonavirulent *P. syringae* control.

When leaves of the parental Col-0 line were inoculated with an extremely high titer of avirulent *P. syringae* (2 x 10^8 cfu/ml), the expected confluent collapse of host cells was observed (Fig. 1) [Kunkel, (1993) supra; Yu, G.-L. et al. (1993) *Mol. Plant-Microbe Interact* **6**:434-443]. However, even at this high pathogen dose, very little cell death above that seen in negative controls was detected in *dnd1* plants (Fig. 1). Separate experiments that used Evans Blue to stain dead or dying cells gave similar results. The autofluorescence assay method was preferred because of greater clarity and less laborious tissue preparation. With the autofluorescence assay, absence of HR cell death in *dnd1* plants was observed in multiple experiments, including experiments that used initial bacterial titers as high as 2 x 10^9 cfu/ml. A slight increase in cell death was observed in ≈5-8% of the *dnd1* leaves inoculated with 2 x 10^8 cfu/ml of avirulent *P. syringae* but only in isolated areas that represented a fraction of the inoculated tissue. Cell death in these small areas was patchy rather than confluent, and similar small patches of cell death could be observed at a lower frequency in control Col-0 plants inoculated with the nonavirulent *P. syringae* strain. No stimulation of cell death by avirulent *P. syringae* could be detected in the vast majority of the inoculated *dnd1* leaves.

To determine whether the absence of the HR in the *Arabidopsis dnd1* mutant is associated with compromised disease resistance, growth of *P. syringae* pv. *tomato* within plants was monitored quantitatively over time [Whalen, M. et al. (1991) *Plant Cell* **3**:49-59]. Pathogenic strains that express an avirulence gene are virulent on plants that do not express the corresponding resistance gene, but their growth is reduced severely on plants which possess the appropriate resistance gene. Fig. 2A shows the growth of *P. syringae* pv. *tomato* expressing *avrRpt2* in wild-type *Arabidopsis* Col-0 (*RPS2/RPS2*), in a Col-0 line lacking functional *RPS2* (*rps2-201/rps2-201*), and in the Col-0 *dnd1* mutant. Despite the absence of the HR, *dnd1* was very similar to wild type in successfully restricting the growth of *P. syringae* expressing *avrRP2*. Strong avirulence and resistance gene-dependent restriction of pathogen growth also was observed in quantitative experiments with *P. syringae* expressing *avrRpm1, avrRps4,* or *avrB* (Fig. 2B). These results demonstrate that extensive HR cell death is not always required for resistance gene/avirulence gene-dependent plant disease resistance.

Having established that *dnd1* plants are resistant to avirulent to *P. syringae* despite the absence of the HR, the response of the *dnd1* mutant to virulent *P. syringae* was examined. Fig. 2B shows the growth of the virulent *P. syringae* pv. *tomato* strain DC3000 (pVSP61) in wild-type Col-0 and in Col-0 *dnd1/dnd1* plants (open symbols). This strain does not trigger gene-for-gene resistance in plants of the Col-0 gentoype [Kunkel, (1993) supra; Whalen, (1991) supra], yet leaf populations of this pathogen strain were reduced 10- to 100-fold in experiments with the *dnd1* mutant. Similar results were obtained in multiple experiments and in studies with the virulent *P. syringae* pv. *maculicola* strain 4326. The *dnd1* plants express a level of resistance to virulent *P. syringae* that is typical of plants exhibiting systemic acquired resistance, induced systemic resistance, or other forms of resistance gene-independent disease resistance [Ryals, J.L. et al. (1996) *Plant Cell* **8**:1809-1819; Pieterse, C.M. et al. (1996) *Mol. Plant-Microbe Interact* **8**:1225-1237]. This broad spectrum resistance phenotype co-segregated with the other *dnd1* mutant phenotypes in all cases tested.

Important to note, Fig. 2B also shows that growth of populations of *P. syringae* that do express *avrRpm1* (closed symbols) was restricted to a much greater extent than was growth of the virulent pathogen strain. A 1,000- to 10,000-fold reduction of pathogen growth was observed if the otherwise virulent *P. syringae* strains DC300 or 4326 expressed avirulence genes *avrRpm1* or *avrRpt2* (Fig. 2B). These experiments demonstrated that gene-for-gene resistance can be induced over and above the weaker resistance gene-independent resistance in *dnd1* plants.

To examine the extent of the lower level resistance to virulent pathogens in the *dnd1* mutant, plants were inoculated with virulent strains of other pathogen species (Lee, J.-M. et al. (1996) *Mol. Plant-Microbe Interact.* **9**:729-735; Bent, A., et al. (1992) *Mol. Plant-Microbe Interact* **5**:372-378; Parker, J.E. et al. (1993) *Mol. Plant-Microbe Interact* **6**:216-224; Parker, J.E. et al. (1997) *Plant Cell* **9**:879-894]. Tobacco ringspot virus spread systemically in only 9% of *dnd1* plants as opposed to 71% for wild-type Col-0. *Xanthomonas campestris* pv. *campestris* and *X. c.* pv. *raphani* (bacteria) only produced mild yellowing on *dnd1* rather than the necrotic lesions produced on Col-0. *Peronospora parasitica* (oomycete) produced three-fold fewer spores on *dnd1* as opposed to Col-0 [3.0 ±2.2 vs. 10.7 ± 3,1 mean ± SE if (spores x 10^3) per leaf]. Microscopy of leaves infected with virulent *P. parasitica* confirmed that restriction of mycelial growth was not associated with HR-like host cell necrosis or autofluorescence. At 3 days postinoculation, mycelia of virulent *P. parasitica* strain Noco2 typically had formed haustoria on 2-10 host cells in *dnd1* plants, whereas in wild-type Col-0 plants a typical mycelium ramified extensively and formed haustoria on 15-30 host cells. Significantly reduced growth of *Erysiphe orontii* (fungus) in *dnd1* plants also has been observed.

Constitutively elevated broad spectrum resistance has been observed previously in a number of contexts, such as in *Arabidopsis cpr, cim, lsd,* and *acd* mutants [Dangl, (1996) supra], in hybrid tobacco lines derived from crosses between disparate *Nicotiana* species [Ahl Goy, et al. (1992) *Physiol. Mol. Plant Pathol.* **41**:11-21], and in plants expressing systemic acquired resistance in response to prior pathogen infection or treatment with salicylic acid or synthetic salicylic acid mimics [Ryals, (1996) supra]. Elevated resistance often is associated with increased expression of pathogenesis-related (PR) genes [Ryals, (1996) supra], and examination of uninoculated *dnd1* plants revealed constitutively increased expression of the PR genes β-glucanase and PR-1 (Figs. 3A and 3B) [Cao, H. et al. (1994) *Plant Cell* **6**:1583-1592; Ausubel, F.M. et al. (1997) *Current Protocols In Molecular Biology* (Wiley, New York)]. Although plants infected by virulent *P. syringae* pv. *tomato* displayed elevated levels of β-glucanase or PR-1 mRNA, inoculation of *dnd1* or wild-type Col-0 with avirulent *P. syringae* expressing *avrRp2* caused an even greater elevation in PR-1 mRNA (Fig. 3C) (25, 33). Similar or more pronounced results were obtained with four distinct RNA sets prepared, blotted, and probed in entirely separate experiments. These results demonstrate, at the level of gene expression, that gene-for-gene signal transduction and defense response activation are functional in *dnd1* plants and are inducible over and above constitutive broad spectrum resistance.

Enhanced PR gene expression and broad spectrum resistance can be induced by elevated levels of endogenous or applied salicylic acid compounds [Ryals, 1996) supra]. We observed constitutively elevated levels of both free salicylic acid and glucoside-conjugated salicylates in *dnd1* plants (Fig. 4). Although salicyclate is likely to be a primary mediator of heightened resistance in *dnd1* plants, the mechanism by which the *dnd1* mutation causes salicylate elevation remains to be discovered.

Plant mutants that display gene-for-gene disease resistance with no HR cell death are not common. However, other *Arabidopsis* mutants that exhibit constitutively elevated resistance have been isolated, such as the *cpr, cim, lsd,* and *acd* mutants [Dangl, (1996) supra; Bowling, S.A. et al. (1997) *Plant Cell* **9**:1573-1584; Bowling, S.A. et al. (1994) *Plant Cell* **6**:1845-1857; Lawton, K. et al. (1993) in *Mechanisms of Defence Responses in Plants*. eds. Fritig, B. & Legrand, M. (Kluwer, Dordrecht, The Netherlands), pp. 422-432]. Accordingly, *dnd1* plants were compared with a number of these lines. In contrast to the *acd* and *lsd* mutants, no lesion-mimic phenotype was observed in *dnd1* mutants when leaf tissue from uninoculated plants was inspected by naked eye, by autofluorescence microscopy as described in Yu, (1993) supra, or after trypan blue staining as described in Parker, J.E., et al. (1993) *Plant J.* **4**:821-831. Genetic complementation tests demonstrated that *dnd1* is a separate locus from the two published *cpr* loci, *CPR1* and *CPR5* (see Examples section). In addition, the *dnd1* mutant apparently does not resemble many of the other unpublished *cpr* or *cim* mutants because the *dnd1* mutant does not exhibit traits observed in preliminary analysis of those mutants such as dominant or semi-dominant behavior, very low fertility, glabrousness, or distorted leaf shape. In particular, previously described *cpr* and *cim* mutants do not display the *dnd* phenotype of gene-for-gene defense with no HR cell death. The *dnd1* mutant does exhibit a dwarf phenotype, as is observed in *Arabidopsis cpr, cim,* and other constitutive PR-expression mutants, but *dnd1* plants otherwise appear normal in their growth and development.

The *dnd* mutants were examined to determine whether they are also resistant to other inducers of cell death. As shown in Fig. 5A-5B and additional experiments, both *dnd1* and *dnd2* mutants exhibited delayed response and reduced sensitivity to Fumonisin B1-induced cell death compared to the wild type *Arabidopsis*, indicating that the *dnd* mutants may have more general suppression of programmed cell death. Fumonisin B 1 is a known inhibitor of ceramide synthase which induces apoptosis in diverse organisms.

To determine the genetic basis of the *dnd1* phenotype, segregation analysis and gene mapping studies were carried out. Crosses of *dnd1* to wild-type Col-0 and No-0 ecotypes yielded F1 individuals that display the wild-type HR^+ phenotype, demonstrating the recessive nature of the mutant phenotype. F2 of a Col-0 x *dnd1* cross segregated 24:7 for HR^+:HR^-, F2 of a No-0 x *dnd1* cross segregated 154:55, and F2 of a reciprocal *dnd1* x No-0 cross segregated 132:45. These data are consistent with a 3:1 ratio (for χ^2 test, P = 0.59, 0.66, and 0.90, respectively), indicating that a single mutant locus controls the observed phenotypes. The reduced rosette size phenotype was also recessive, and absolutely co-segregated with the HR^- phenotype in these and all other F2 plants analyzed. The gene symbol *DND1* was chosen for this locus, reflecting the mutant phenotype of *D*efense with *N*o HR cell *D*eath. PCR-based microsatellite and cleared amplified polymorphic sequence genetic markers were used to map the mutated locus. No linkage was detected except to markers for the top arm of chromosome 5. Fine-structure mapping with 536 F2 individuals from No-0 *dnd1* crosses yielded only six recombinant chromosomes between *dnd1* and CHS1. These experiments placed *DND1* within the ≈1.6-cM interval between CHS1, and nga 106 and a different 11 recombinant chromosomes between *dnd1* and CHS1. These experiments placed *DND1* within the ≈1.6-Cm interval between CHS1 and nga 106 on the upper arm of *Arabidopsis* Chromosome 5. This location defines a map position that has not been associated previously with defense-related genes.

Genetic mapping data suggested that the *DND1* locus resides to the north and close to marker pCIT1243 on the top of *Arabidopsis* chromosome 5. In order to isolate the clone for the *DND1* gene, four contiguous BACs (8M21, 3H2, 22L1 and 23B17) were generated which subsequently used to generate a redundant cosmid library. The detailed techniques for creating BACs, cosmid library, and the use of RFLPs are well known in the art and can be found in Ausubel, (1997). Once a small number of cosmids were identified to span the *DND1* locus region, each clone was tested for the capacity to functionally complement the *dnd1* mutation. This was accomplished by transforming mutant *dnd1* plants with each of the cosmids via *Agrobacterium*-mediated transformation and screening transformants for reversion to wild-type characteristics. To simplify this process, putative transformants were initially screened solely on the basis of size. Because all known phenotypes of *dnd1* mutants appear to be tightly linked, complementation of dwarf size was considered to represent genetic complementation of the *DND1* locus. In general *dnd1* plants exhibited a significantly lower transformation rate, relative to wild-type Col-0 (~ .001% transformants per total number of seeds tested compared to ~.2-.5% for Col-0). The transformants were planted to soil and after 2-3 weeks analysed for the size. T1 plants from the three cosmids (1A8, 1H2 and 1H3) exhibited size similar to that of wild-type controls and overlapped to the same region of BAC 3H2 (see Fig. 9). In summary, complementation data delimited the location of the *DND1* locus and demonstrated that the gene encoded in the region is responsible for the loss of function, i.e. dwarfism, in the *dnd1* plants.

To confirm genetic complementation of the *DND1* locus further, HR assays and bacterial growth curves were performed on T2 plants from the three size complementing cosmids to verify reversion to wild-type defense responses. Because plants transformed with *Agrobacterium* are typically hemizygous for the transgene, it was not surprising to observe T2 plants from each of the cosmids segregating 3:1 (wild-type:dwarf) for size: 1A8 (23:6), 1H2(32:10), and 1H3 (29:13). Thus, these segregating T2 populations contain *dnd1* plants complemented by the cosmid transgene, as well as noncomplemented mutant *dnd1* plants.

As expected, T2 plants of wild-type size exhibited defense responses similar to that of Col-0, while T2 plants of dwarf stature displayed defense responses comparable to that of *dnd1* plants. The trademark phenotype of *dnd1* is the absense of a HR while challenged with avirulent Psg. However, *dnd1* plants transformed with cosmid 1A8 or 1H2, that were of wild-type size, displayed a strong HR response to Psg R4(*avrRpt2*$^+$) (Fig. 10). Conversely, dwarf T2 plants were defective in HR cell death indicating that these dnd1 plants did not contain a complementing cosmid transgene (Fig. 10).

Another defense response characteristic of *dnd1* mutation is elevated resistance to virulent pathogens. T2 plants transformed with cosmid 1H3 that were wild-type in size were susceptible to Pst DC3000 (i.e. their response mirrored that of Col-0). As shown in Fig. 11, a day three growth analysis of dwarf T2 plants provided data to indicate that these plants retained elevated resistance characteristic of the *dnd1* mutation. Thus these plants did not contain a complementing cosmid transgene.

A series of subcloning and subsequent functional testing as described above yielded the subclones and complementation data summarized in Figure 12. Note in particular that the generic region encoding *DND1* was closely delineated by successful complementation with subclones 18B and 27.1, and by the failure to complement with 56.2 or 61.1. Subcloning also yielded a clone (17.1) of 5.2 kb in length. A nucleotide BLAST search with partial sequence data generated from the clone yielded a perfect 470 bp match to *Arabidopsis thaliana* cyclic nucleotide-gated cation channel *AtCNGC2* mRNA (Accession ATY 1628). This cDNA was obtained by screening an *Arabidopsis* EST database with the cyclic nucleotide binding domain of a mammalian ion channel [Kohler et al. (1998) *The Plant Journal* **18**(1):97-104]. It has a 2178 bp open reading frame that encodes a 726 amino acid protein marked by a cyclic nucleotide binding domain in the C-terminus, a putative calmodulin binding site, and hydrophobic regions at the N-terminus (Figs. 6 and 7). Sequencing of the genomic DNA spanning the *AtCNGC2* cDNA revealed that *DND1* (*AtCNGC2*) is a 3327 bp gene composed of 8 exons (Fig. 6). Subsequent cloning and sequencing identified the nature of the *dnd1* mutation to be a G to A transition creating a premature stop codon at amino acid 120 (Fig. 6).

The *dnd2* mutant was analyzed similarly according to the procedure established for characterizing the *dnd1* mutant as disclosed herein and found to be similar to the *dnd1* mutant in most aspects; whole plant phenotypic data for *dnd 1* were representative of similar data collected for *dnd* 1 plants. The *dnd2* mutation was recessive to wild type, and homozygous *dnd2/dnd2* mutant plants exhibited an extreme reduction in the extent of HR cell death in response to avirulent P. syringae. The *dnd2* mutant plants also exhibited a dwarf (smaller-sized) plant growth habit, constitutively elevated levels of free- and conjugated-salicylic acid in leaf tissues, and a constitutive broad spectrum defense phenotype that resembles plants induced for systemic acquired resistance. The phenotypes of *dnd2* mutant plants cosegregated as a single Mendelian locus in the F2 progeny of crosses to wild type. However, it was noted that *dnd2* plants do differ from *dnd1* plants in one phenotypic respect; they tend to become chlorotic or yellowed at the leaf tips and distal lateral margins of leaves at a time when most leaves of wild type *Arabidopsis* or *dnd1 Arabidopsis* do not show this yellowing.

While *DND1* maps to the upper arm of *Arabidopsis* chromosome 5, *DND2* maps to the lower arm of that chromosome 5. The *DND2* gene maps to the genetic interval flanked by the available PCR based, polymorphism-detecting genetic markers nga129 and LFY3 (www.arabidopsis.org).

Further genetic mapping of the *DND2* locus using F2 individuals and F3 families from a cross of Col-0 *dnd2-1/dnd2-1* to ecotype No-0 has refined the site of the *DND2* locus to the genetic interval between g4130 and K19P17. This corresponds to a genetic size approximately 2.5 centiMorgans, spanned by six overlapping BAC clones, covering approximately 400 kb of *Arabidopsis* genome. It was noted that the *Arabidopsis* genome within this interval has recently been sequenced, annotated, and released to Genbank. A survey of the genes encoded within this interval revealed a putative cyclic nucleotide-gated ion channel (*CNGC*) encoding gene, termed *AtCNGC1* (Kohler and Neuhaus 1998, supra).

PCR primers were designed to amplify the segment of *Arabidopsis* ecotype Col-0 wild type genomic DNA shown in Fig.13 and SEQ ID NO:4. The primer sequences were: MFH813.9X (SEQ ID NO:7), 5'-ATCCGCTCGAGTGATTGGTTTCGTCTTGTCC-3'; and MFH819.9B (SEQ ID NO:8), 5'-TTCGCGGATCCTATGCACTGTGCCTGTGTGA-3'. The resulting PCR product DNA spanned the entire *AtCNGC1*-coding sequence (see Fig. 14 and SEQ ID NO:5) as well as roughly 2 kb of upstream DNA (putative promoter region) and roughly 0.5 kb of downstream DNA (putative terminator region). High-fidelity DNA polymerase (Taq polymerase, Stratagene Co. La Jolla, CA) was used in the polymerase chain reaction together with the above primers and template to generate the expected product. This product was cloned into the *Agrobacterium*/plant transformation-competent plasmid vector pCLD04541 [Jones, J.D.G. et al. (1992) *Transgenic Research* **1**:285-297]. The resulting products (from three independent PCR reactions), named pACol-01-1a, pSCol-07-23a, and pZCol-08-27c, were moved in to *Agrobacterium tumefaciens* and used to genetically transform *Arabidopsis* Col-0 *dnd2-1/dnd2-1* plants, using the "floral dip" method [Clough and Bent (1998) *Plant J.* 16:735-743]). Putative transformants were identified by selection on kanamycin plates using standard methods. These putative transformants were then transplanted to soil while still very young (roughly ten days old). After growth for an additional few weeks, it became apparent that with all three plasmid constructs, the transformed *dnd2* mutant plants had been phenotypically complemented and resembled wild-type rather than *dnd2*. As in the successful positional cloning of *DND1*, this was initially determined by observation of plant size [Clough et al. (2000) *Proc.Natl.Acad. Sci.* (USA) in press]. Control *dnd2* plants transformed with pCLD04541 vector that does not contain *AtCNGC1*-spanning DNA did not exhibit phenotypic complementation (see Fig. 16).

In summary, the *DND1* and *DND2* genes discovered initially by their phenotypic characteristics, i.e., enhanced disease resistance and suppression of HR cell death, both encode protein products with clear similarity to mammalian and other metazoan cyclic nucleotide-gated ion channels [Kohler and Neuhaus (1998) Supra; Kohler et al. (1999) *Plant J.* 18:97-104; Leng et al. (1999) *Plant Physiol* 121:753-761]. cDNAs derived from these loci have been studied by other groups. Recent studies by Leng et al. demonstrated that the product of *AtCNGC2* is indeed a functional ion channel that is gated by cyclic nucleotides. However, the present invention is the first disclosure that makes the critical connection between cyclic nucleotide-gated ion channel genes and the disease resistance/suppression of cell death functions of the mutated *DND* genes. Accordingly, this invention provides methods of making disease resistant plants by manipulating either a *DND* gene (or gene product) or a cyclic nucleotide-gated ion channel gene (or gene product).

The discovery of the *AtCNGC2/DND1* and *AtCNGC1/DND2* genes as regulators of disease resistance together with availability of the genomic sequence information make it possible that plant disease resistance or cell death can be manipulated by the recombinant DNA technology well known in the art. For example, one skilled in the art can use the nucleotide sequences of the *AtCNGC/DND* genes disclosed herein to isolate related genes in other plants. The *DND* 1 and 2 genes share about 46% sequence identity at the nucleotide level in the coding region. It is likely that a functional or structural homolog of the *AtCNGC2/DND1* and *AtCNGC1/DND2* genes would share similar sequence homology. Once identified, these genes can be employed to improve disease resistance. The *CNGC/DND* protein or a homolog thereof can be modified by substitution of amino acid residues, deletions, additions, and the like. Mutants generated may exhibit diverse phenotypes in addition to varying degrees of pathogen resistance. A mutant (or mutants) exhibiting an enhanced disease resistance without a dwarfed stature can be isolated. Methods for mutagenesis and nucleotide sequence alterations are well known in 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.

Alternatively, the disease resistance can be enhanced by inactivating or downregulating the *CNGC/DND* gene or a homolog thereof. The *DND1* genomic sequence shown in Fig. 6 contains about 1.6 kb 5' flanking sequence in addition to introns and exons, and about 700 nucleotides of 3' flanking region. The *DND2* genomic sequence shown in Fig. 13 contains about 2 kb 5' flanking sequence and about 0.5 kb 3' flanking sequence in addition to the coding sequence for *AtCNGC*1. The flanking sequences surrounding the gene generally contain various regulatory sequences which control expression of the gene, either transcriptionally or translationally. Therefore, the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene expression can be down-regulated or inactivated by either transcriptionally or translationally. Similarly, one skilled in the art can derive any antisense molecule based on the sequences shown herein including the splice sites (i.e. intron-exon junction) to inactivate or down-regulate the *CNGC/DND* gene. Sense-strand suppression, virus-induced gene silencing, double-strand RNA and other inactivation methods are also applicable [Hamilton and Baulcombe (1999) *Science* **286**:950-952; Somerville, C. et al. (1999) *Science* **285**:380-383; Jorgensen, et al. U.S. Patent No. 5,283,184]. The flanking sequences containing regulatory elements for transcription can also be used to identify compositions which inhibit *CNGC/DND* gene expression.

The *DND1* and *DND2* genes are highly related as evidenced by the sequence homology (~46% identity at the nucleotide level). Kohler et al (1999) reported a gene family of 6 putative CNGCs in *Arabidopsis thaliana* which share significant structural homology. One skilled in the art can easily utilize the nucleotide sequences encoding the *DND1* and *DND2* genes provided herein to isolate additional potential disease resistance genes.

The nucleotide sequences encoding the *AtCNGC2/DND1* and *AtCNGC1/DND2* can be utilized to isolate homologous genes from other plants including sorghum, Brassica, wheat, tobacco, cotton, barley, sunflower, cucumber, alfalfa, soybeans, sorghum etc. Coding sequences from other plants may be isolated according to well known techniques based on their sequence homology to the *AtCNGC2/DND1* or *AtCNGC1/DND2* coding sequences set forth herein SEQ ID NOs: 2 and 5. In these techniques all or part of the known coding sequence is used as a probe which selectively hybridizes to other disease resistance coding sequences present in genomic or cDNA libraries from a chosen organism, or genomic sequence, or coding sequences are used to design PCR primers for the same purpose. Alternatively, homologous genes can be identified from the EST or genomic sequence databases using *AtCNGC2/DND1* or *AtCNGC1/DND2* genomic or cDNA sequences. Similarly, searching can utilize the entire *AtCNGC2/DND1* or *AtCNGC1/DND2* gene or derived amino acid sequence, or subdomains thereof. Methods for similarly searching can be found in Brenner, S. and Lewitter, F., editors (1998) Trends Guide to Bioinformatics., Elsevier Science Ltd., Oxford, U.K. Identification of *AtCNGC2/DND1* or *AtCNGC1/DND2* and their homologs in other plants may facilitate identification of effector genes that interact with *AtCNGC2/DND1* or *AtCNGC1/DND2* or their homolog gene or gene product; or the identification of effector chemicals or other interventions that alter *AtCNGC2/DND1* or *AtCNGC1/DND2* function in a desirable fashion. A detailed protocol for these experiments including hybridization screening of plated DNA libraries can be found in Sambrook et al., Molecular Cloning, eds., Cold Spring Harbor Laboratory Press (1989); Ausubel, F.M. et al. (1997) "Current Protocols in Molecular Biology" Wiley, New York.

For example, hybridization of such sequences may be carried out under conditions of reduced stringency, medium stringency or even high stringency 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 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 disease resistance genes disclosed herein in a standard hybridization assay.

Mutation of a cyclic nucleotide-gated ion channel gene in plants other than *Arabidopsis* can be used in a conventional plant breeding program to introduce a *dnd* phenotype into an elite variety. Such mutations can be identified as described herein for *Arabidopsis*. The breeding is facilitated by identifying one or more markers linked to the *DND* gene. Such markers can include conventional markers or molecular markers such as RFLP or SSR markers. For example, SSR (simple sequence repeat) markers have been mapped for the entire soybean genome and are publicly available from USDA (see http:SoyBase.agron.iastate.edu) or from Research Genetics Inc., Huntsville, AL. Conventional mapping methods are used to identify one or more SSR markers linked to the *DND* locus. Similar molecular markers are available for most agronomic crops. By conventional breeding, a suitable *DND* mutant allele can be introgressed into a desired commercial soybean line by following an appropriate linked SSR marker during crossing and backcrossing, as is known in the art. The same process outlined above can be used, with appropriate markers, for crossing *DND* mutations into other plant varieties.

The methods of the present invention and methods known in the art 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. *Proc. Natl. Acad. Sci. USA*. **83**:5602-5606]; *Agrobacterium* mediated transformation [Hinchee et al. *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; 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; and Tomes et al. "Direct DNA transfer into intact plant cells via microprojectile bombardment" In Gamborg and Phillips (Eds.) Plant Cell, Tissue and Organ Culture: Fundamental Methods, Springer-Verlag, Berlin (1995); Hooydaas-Van Slogteren & 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., pp. 197-209; Longman, NY (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 which 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 the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that the subject phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure the desired phenotype or other property has been achieved.

Efficient regeneration of plants from single cells or protoplasts is essential in the genetic manipulation of plants using various gene transfer technologies. The detailed protocols for such procedures can be found in the following references: Li, H.Q. et al., (1996) *Nat. Biotechnol.* **14**(6):736-740; Ghosh Biswas, G.C. et al. (1994) *J. Biotechnol.* **32**(1):1-10; Datta, S.K. et al. (1992) *Plant Mol. Biol.* **20**(4):619-629; and Lorz, H. et al. (1979) *Planta. Med.* **36**(1):21-29.

As noted earlier, the nucleotide sequences of the invention can be utilized to protect plants from disease, particularly those caused by plant pathogens. Pathogens of the invention include, but are not limited to, viruses or viroids, bacteria, fungi, and the like. Specific examples of these pathogens include, but are not limited to, the pathogens listed in Table I.

The identification of the *DND1* and *DND2* genes as cyclic nucleotide-gated channel genes in *Arabidopsis* offers additional means to identify compositions which can enhance disease resistance in plants. Plant tissue cultures and recombinant plant cells containing the proteins and nucleotide sequences of *CNGC/DND* gene, or transgenic cells of other species such as *Eschericha coli* or *Saccharomyces cerevisae* or *Xenopus laevis* that express the *CNGC/DND* protein or the purified *CNGC/DND* protein may be used in an assay to screen compositions which inhibit the function of the cyclic nucleotide-gated channel protein. Such an assay is useful as a general screen to identify compositions which inhibit *AtCNGC2/DND1* or *AtCNGC1/DND2* protein activity. The detailed assay protocol for measuring the channel activity can be found in Leng et al. (1999) *Plant Physiol.* **121**:753-761. A composition that results in less channel activity upon addition to the assay, compared to that of control, is defined as an inhibitor. If such a composition is found, it would be useful to enhance disease resistance in plants.

As discussed, the genes of the invention can be manipulated to enhance disease resistance and/or cell death in plants. In this manner, the expression or activity of the *AtCNGC2/DND1* (or *AtCNGC1/DND2*) or other disease resistance genes can be altered. Such means for alteration of the gene include co-suppression, antisense, mutagenesis, alteration of the sub-cellular localization of the protein, etc. In some instances, it may be beneficial to express the gene from an inducible promoter, particularly from a pathogen inducible promoter or from a tissue-specific or growth-stage-specific promoter, or by a chemical-spray induced fashion (see U.S. 5,689,042, U.S. 6,008,436, U.S. 5,589,622, and U.S. 5,789,214). 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. (1983) *Neth. J. Plant Pathol.* **89**:245-254; Uknes et al. (1992) *The Plant Cell* **4**:645-656; and Van Loon (1985) *Plant Mol. Virol.* **4**:111-116.

Plants homozygous for the *dnd1* or *dnd2* mutation exhibit substantial suppression of "hypersensitive response" (HR) cell death, a form of localized cell death associated with "gene-for-gene" plant disease resistance and also associated with some instances of pathogen-induced necrosis as part of disease damage or susceptibility. This cell death is beneficial to the plant in some instances but is deleterious in others. Plant cell death can be partially or completely controlled by similar modifications of the *AtCNGC2/DND1* or *AtCNGC1/DND2* gene or homolog thereof as disclosed in the present invention.

EXAMPLES

The following examples are provided for illustrative purposes, and are not intended to limit the scope of the invention as claimed herein. Any variations in the exemplified articles which occur to the skilled artisan are intended to fall within the scope of the present invention.

Example 1. Inoculations with *P. syringae*.

Original mutants and their progeny were tested for the HR by pipet inoculation of individual leaves with *P. syringae* pv. *glycinia* Race 4 pV288 (*asvrRp2*$^+$) or Race 4 p VSP61 (no *avr* gene) at ≈2 x 10^8 colony forming units (cfu)/ml (19, 20). Additional *P. syringae* strains used to test for gene-for-gene HR included *P. syringae* pv *glycinia* Race 4 pAvrRpml (*avrRpml*$^+$) and Race 4 pVB01 (*avrB*$^+$) [Kunkel, (1993) supra; Bisgrove, (1994) supra]. Positive and negative *Arabidopsis* controls included the use of wild-type Col-0, Col-0 *rps-201/rps2-201,* and Col-0 *rpm/rpml ("rps3-1")* mutants [Kunkel, (1993) supra; Bisgrove, (1994) supra.] For bacterial growth experiments and for gene expression studies, *P. syringae* pv. *tomato* strain DC3000 and *P. syringae* pv. *maculicola* strain 4326 were used with the above plasmids or with pKec218 (*avrRps4*^{4}) [Hinsch, M. et al. (1996) *Mol. Plant-Microbe Interact.* **9**:55-61]. Quantitative determinations of bacterial growth in leaves were performed by dilution plating of homogenized leaf tissue on selective media, as described in Whalen, (1991) supra.

Example 2. Mutant Screen and Crossing.

Arabidopsis thaliana ecotype Col-0 seeds were mutagenized with ethyl methane sulfonate; M2 populations were obtained from Lehle Seeds (Round Rock, TX). To test for activation of the HR, *P. syringae* pv. *glycinia* Race 4 pV288 (*avrRpt*$^+$), at a concentration of ≈2 x 10^8 cfu/ml in 10 mM $MgCl_2$, was introduced by vacuum infiltration into leaf mesophyll tissue of ≈11,000 M2 seedlings. Leaves were observed 24 and 40 h after infiltration, and plants with reduced, delayed, or no leaf collapse were saved for further analysis. Lines of potential interest were crossed with the wild-type Col-0 parent to initiate backcrossing and with ecotype No-0 to initiate genetic mapping. For complementation tests, *Arabidopsis* Col-0 *dnd/dnd1* plants were crossed to homozygous *cpr1* and *cpr5* mutants, which also display a reduced rosette size [Bowling, S.A. et al. 1997) *Plant Cell* **9**:1573-1584; Cao, (1994) Dominance/recessiveness and genetic complementation were deduced by observation that all F1 plants were silt-type in appearance and displayed the HR after inoculation with *P. syringae* pv *glycinia* Race 4 pV288.

Example 3. Microscopy.

To monitor HR cell death at the cellular level, pipet infiltration was used to introduce*P. syringae* pv. *glycinia* Race 4 pV288 (*avrRpt*$^+$) or Race 4 pVSP61 (no *avr* gene) into 40-=70% of the mesophyll space of individual leaves, at the bacterial concentrations indicated. Leaves were removed from plants after 24 h, fixed in 2% formaldehyde, 5% acetic acid, and 40% ethanol for 30 min, and then cleared sequentially in 50% ethanol and 95% ethanol [Yu, (1993) supra]. Leaf parenchyma cells then were examined for HR-associated autofluorescence by using fluorescence microscopy with a fluorescein filter set (Ex 495 $\pm$ 20 nm, Em $>$ 505 nm [Klement, (1990) supra]. Alternatively, Evan's Blue (Sigma) was infiltrated into leaves as a 1% aqueous solution 22-26 h after pathogen inoculation [Klement, (1990) supra]. After at least 10 min of staining, leaves were removed from plants, a portion of the epidermis was peeled back, and leaves were rinsed in H_2O, mounted in H_2O, and observed by light microscopy. Leaf areas damaged by physical handling were not considered when evaluating the proportion of dead and living cells.

Example 4. Genetic Mapping.

F2 populations from a No-0 x Col-0 *dnd1/dnd1* cross were used for mapping. The HR phenotype was assessed visually 24 and 48 h after pipet inoculation of leaves with *P. syringae* pv. *glycinia* Race 4 pV288 (*avrRpt*$^+$) resuspended to $\approx 1 \times 10^8$ cfu/ml in 10 mM $MgCl_2$. Informative F2 lines were retested for HR in selfed F3 families. PCR-based cleaved amplified polymorphic sequence and microsatellite markers were used as described in Bell, C.J. et al. (1994) *Genomics* **19**:137-144; and Konieczny, A. et al. (1993) *Plant J.* **4**:403-410; a set of 17 markers spanning all five *Arabidopsis* chromosomes was used for initial linkage analysis.

Example 5. Inoculations with Other Pathogens.

Tobacco ringspot virus grape strain was applied to plants, and virus multiplication was monitored by using ELISA as described in Lee, (1996) supra. *Xanthomonas campestris* pv. *campestris* strain 2669 [Parker, J.E. et al. (1993) *Mol. Plant-Microbe Interact.* **6**:216-224] were applied at a concentration of ≈ 1 x 10^7cfu/ml and monitored as described in Parker, (1993) supra. *Peronospora parasitica* isolate Noco2 was applied and monitored as described in Parker, J.E. et al. (1997) *Trends Biochem. Sci.* **22**:291-296. For all experiments, *Arabidopsis* ecotype Col-0 served as a susceptible control for pathogen multiplication and virulence.

Example 6. Gene Expression Studies.

P. syringae pv. *tomato* strains DC3000 (pV288) or DC3000 (pVSP61) were introduced into leaf mesophyll of intact plants by vacuum infiltration (as above), typically at a dose of 5 x 10^4 cfu/ml. Total RNA was extracted from leaf material and equal quantities of RNA from each sample were separated in agarose-formaldehyde gels, blotted, and hybridized with ^{32}P-radiolabeled probe essentially as described in Ausubel, (1997) supra. DNA probes were from Cao et al. [Cao, (1994) supra]. Hybridization was quantified by using a storage phosphor imaging system according to the manufacturer's instructions (Molecular Dynamics). Signal for PR-1 or β-glutanase in each lane was normalized to the control β-ATPase signal for that lane to correct for slight differences in gel loading, and normalized signals then were divided by the signal for the Col-0/no-pathogen sample to establish a relative scale.

Example 7. Salicylic Acid Determinations.

Salicylic acid determinations were performed as described in Uknes, S. et al. (1993) *Mol. Plant-Microbe Interact.* **6**:692-698 on leaf material from uninoculated 6-week-old plants.

Example 8. Functional Complementation of the *dnd1* phenotype.

Tri-parental mating: In order to transform the cosmids into *Arabidopsis* for complementation studies, the cosmids were put into *Agrobacterium*. Members of the cosmid library were transferred to *Agrobacterium tumefaciens* strain GV3101 via a tri-parental mating. Liquid cultures were prepared for each of the parents: GV3101 (pMP90), *E. coli* strain HB101 containing the mating helper plasmid pRK2013, and the cosmid-bearing *E. coli* XL-1 donor.

Cultures were spotted on LBA media (no antibiotics) such that an approximate 5:1:1 ratio of recipient, helper, and donor was achieved within a single spot for each cosmid. These mating spots were grown overnight at 28°C. The next day each mating spot was re-streaked onto low-salt LB media (10g tryptone, 5 g yeast extract, and 5 g NaCl/liter) + tetracycline (2.5 μg/ml) + rifampicin (100 μg/ml) + gentamycin (50 μg/ml) and grown at 28°C for two days. Colonies were picked from these plates and re-streaked unto low-salt LBA (1.5% agar) containing rifampicin (100 μg/ml) and kanamycin (25 μg/ml) to select for *Agrobacterium* colonies containing a cosmid vector.

Arabidopsis transformation: Mutant *dnd1* plants were transformed with *Agrobacterium* harboring cosmid clones via the floral dip method (Clough and Bent, 1998). bacterial cultures (150 ml) of each cosmid were grown overnight at 28°C in low-salt LB +kanamycin (25 μg/ml), spun at 6,000 rpm for 15 minutes. Bacteria were resuspended in 5% sucrose spiked with .05% surfactant Silwet L-77 (Osi Specialities, Inc.). Mutant *dnd1* plants were grown in 3.5 inch pots with mounded soil fettered with tulle under 8-15 hr. light in the greenhouse until they displayed primary bolts. Plants were dipped into the *Agrobacterium* solution for 2-5 sec. and then placed under a dome overnight. At this time, plants were moved to 15 hr. light. Seeds were harvested 3-5 weeks later, once the siliques were brown and thoroughly dry. After 2-3 days of additional drying in 1.5 ml micro-centrifuge tubes on the lab bench, seeds were sterilized either by liquid or vapor-phase sterilization as described (Appendix 4). Sterilized seeds were re-suspended in sterile .1% agarose and plated on kanamycin (50 μg/ml) selection plates. Typically, ~3000 seeds were plated per 150 x 15 mm petri plate. After 7-10 days of growth under 24 hr. light, kanamycin resistant seedlings with green leaves and well-established root systems were deemed putative transformants and were transplanted to soil for further analysis. Because *dnd* mutants are ~100X more recalcitrant to transformation than wild-type, several plates of seeds (sometimes 5-10) were screened in order to obtain a few putative transformants.

After putative transformants were obtained and transplanted to soil, they were grown for an additional 3-4 weeks in a growth chamber under 8 hr. light. At this time, the sizes of individual transformants were compared to that of a wild-type Col-0 control (line A21, a vector control transformant that is wild-type size and kanamycin resistant), that was similarly selected on kanamycin plates and transplanted to soil. Putatively complementing cosmids were identified based on size and were allowed to self and T2 seeds were harvested from these plants for further analysis.

Bacterial Growth Curves: In addition to affecting plant size, the *dnd1* mutation also affects pathogen growth and the ability to produce a HR in response to avirulent pathogen. To verify that cosmids complementing the dwarf phenotype of *dnd1* actually complemented the *DND1* locus, reversion of these other characteristic phenotypes of *dnd1* were also examined in these plants. Growth curves were performed on T2 plants transformed with a complementing cosmid, as well as on Col-0 and *dnd1* controls. Plants were vacuum-infiltrated with a *p.s.* pv. *tomato* DC3000 (Pst DC3000) carrying either *avrRP2* (pV288) or no *avr* gene vector only). Approximately 5 x 10^4 cfu/ml bacteria were used for each inoculation (O.D.$_{600}$+.005). This level of pathogen effectively mimics the low pathogen levels that occur during natural infections. For each sample, two leaf discs were taken from each of two plants, in triplicate, using a #1 cork borer. Thus, for each plant/pathogen combination 12 leaf discs were sampled per time point. Samples were collected at 0, 2, and 4 days post inoculation (or just at 3 days). Leaf discs were harvested into a 1.5 ml micro-centrifuge tubes with 200 μl 10 mM $MgCl_2$, ground with a pestle, and diluted serially onto NYGA (5g Bacto-peptone, 3 g yeast extract, 20 ml glycerol, and 15 g agar/liter) + rifampicin (100 μg/ml) + cycloheximide (50 μg/ml), then grown for two days at 28°C. Colonies were counted and the data was analyzed using Sigma Plot (Jandel Scientific, CO).

HR Assay: HR assays were performed on T2 from two complementing cosmids, in addition to Col-0 and *dnd1* controls. Inoculation with high levels of *P.s. glycinia* Race 4 (Psg R4) (obtained from N.T. Keen, Univ. of California-Riverside) carrying *avrRpt2* induces the HR (visible leaf collapse) in incompatible reactions. Plants were inoculated with 2 x 10^8 cfu/ml (O.D.$_{600}$ =.2) bacteria with a syringe and were scored for visible leaf collapse 24 hours after inoculation. The severity of HR was rated on a 0-5 scale (0 = no collapse, 5 = total collapse). For each plant, three leaves were inoculated with Psg R4 (pV288) (with *avr* gene) and one leaf was inoculated with Psg R4 (pVSP61) (no *avr* gene).

Example 9. Modification of Soybean Plants to enhance Disease Resistance and/or reduced Cell Death.

As one example of a use of the present invention, soybean plants can be engineered to exhibit enhanced disease resistance and/or reduced cell death following infection by a pathogen. A soybean DNA sequence encoding a cyclic nucleotide-gated ion channel homologous to one of the DNA sequences described herein can be obtained from information and materials available in the art, without undue experimentation. Information currently available in genomic sequence databases include EST DNA sequences for cDNAs isolated from soybean. Recently, an EST clone (Genbank Accession AW 781088) was identified as a putative CNGC of soybean. Similarly, multiple EST clones have been identified to encode putative CNGC proteins of other plant species including lotus japonicus, tomato, cotton, and watermelon. Using computer-assisted methods, one skilled in the art can derive a probable amino acid sequence encoded by a given cDNA. A researcher can readily identify within sequence databases a soybean DNA sequence that encodes a cyclic nucleotide binding domain or other derived amino acid sequence motifs characteristic of cyclic nucleotide-gated ion channels. The complete DNA sequence for that cDNA, or for the corresponding region of soybean genomic DNA, is then determined, to complete the identification of the sequence as a *DND*/cyclic nucleotide-gated ion channel gene. An expression cassette is constructed for pathogen-induced expression of an antisense or sense gene. For this purpose, many different pathogen-induced genes can serve as the source of a suitable promoter. For example, the promoter region of the pathogenesis-induced soybean PR-1 gene [Genbank accession AF136636, see also Ryals et al. (1996) *Plant Cell* **8**:1809-1819; Raymond et al. (2000) *Plant Cell* **12**:707-720] or another infection-induced promoter, is fused to a small (25-100 bp), medium (101-500 bp) or large (501 bp to full gene-length) segment of the soybean *DND* gene, in sense or antisense-orientation relative to the promoter [Hamilton and Baulcombe (1999) supra; Jorgensen, et al. U.S. Patent 5,283,184; and, Bridges et al., U.S. 5,073,676]. This is followed by a standard transcriptional terminator such as the *Agrobacterium tumefaciens* nopaline synthase 3' terminator region. Using methods well-known to skilled artisans, the PR-1 promoter/antisense *DND*/nos terminator DNA or PR-1 promoter/sense *DND*/nos terminator DNA construct is placed in a vector suitable for biolistic or *Agrobacterium*-mediated transformation of soybean, and then used to transform an agriculturally suitable soybean variety. Transformants are identified by the use of a co-transformed marker gene, using either a selectable marker such as *kanamycin-resistance*, or a screenable marker such as GUS. Transformants are regenerated following techniques known in the art, to produce mature plants. Fertile productive transgenic soybean lines carrying these DNA constructs are thereby created and identified. Plants are tested for pathogen-inducible expression of the PR-1 promoter/antisense *DND*/nos terminator DNA or PR-1 promoter/sense *DND*/nos terminator DNA construct. Plants can be further tested for transcriptional or translational silencing of expression of the endogenous soybean *DND*/cyclic nucleotide-gated ion channel gene. The silencing may arise only in infected tissues, or may arise systemically throughout much of the plant, and may arise due to a variety of molecular mechanisms. Resistance to pathogens or pathogen-induced cell death can be assayed in the transgenic plants. Resistance may occur locally at the site of infection, or may extend systemically to many other portions of the infected plant, and may arise due to a variety of molecular mechanisms. Note that, in keeping with the epidemiology of many plant diseases, initial infections will often occur at a limited number of sites on the plant, so that induction of resistance at an early stage after infection can reduce the spread of infection to other sites on the infected plant and can also reduce the spread of pathogen to other plants.

As a second example of the present invention, soybean plants are engineered to exhibit enhanced disease resistance and/or reduced cell death induced by treatment with an inducing chemical. A soybean *DND*/cyclic nucleotide-gated ion channel gene can be identified by the methods described in the previous paragraph or by other methods discussed herein. DNA constructs are created that contain a chemically inducible promoter such as that disclosed by Ryals et al. U.S. patent 5,789,214 fused to a small (25-100 bp), medium (101-500 bp) or large (501 bp to full gene-length) segment of the soybean *DND* gene, in sense or antisense-orientation relative to the promoter [Hamilton and Baulcombe (1999) supra; (Jorgensen, et al. U.S. Patent 5,283,184; Bridges, et al. supra]. This is followed by a standard transcriptional terminator such as the *Agrobacterium tumefaciens* nopaline synthase 3' terminator region. Using methods well-known to skilled artisans, the promoter/antisense *DND*/nos terminator DNA or the promoter/sense *DND*/nos terminator DNA construct is placed in a vector suitable for biolistic or *Agrobacterium*-mediated transformation of soybean, and then used to transform an agriculturally suitable soybean variety. Identification and regeneration of transformants is carried out as described previously. Fertile productive transgenic soybean lines carrying this DNA construct are thereby created and identified. Plants are tested for chemically-inducible expression of the promoter/antisense *DND*/nos terminator DNA or the promoter/sense *DND*/nos terminator DNA construct. Plants can be further tested for transcriptional or translational silencing of expression of the endogenous soybean *DND*/cyclic nucleotide-gated ion channel gene. The silencing may arise only in infected tissues, or may arise systemically throughout much of the plant, and may arise due to a variety of molecular mechanisms. Resistance to pathogens or pathogen-induced cell death can be assayed in the transgenic plants. Resistance may occur locally at the site of infection, or may extend systemically to many other portions of the infected plant, and may arise due to a variety of molecular mechanisms. Note that, in keeping with the epidemiology of many plant diseases, initial infections will often occur at a limited number of sites on the plant and on a limited number of plants in a given field, so that induction of resistance at an early stage after the initial infection can reduce the spread of infection to other sites on the infected plant and can also reduce the spread of pathogen to other plants. Induction of resistance in plants by chemical treatment prior to infection can reduce the susceptibility to disease of at-risk plants prior to the occurrence of infections.

Techniques and agents for introducing and selecting for the presence of heterologous DNA in plant cells and/or tissue are well-known. Genetic markers allowing for the selection of heterologous DNA in plant cells are well-known, e.g., genes carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin. The marker allows for selection of successfully transformed plant cells growing in the medium containing the appropriate antibiotic because they will carry the corresponding resistance gene. In most cases the heterologous DNA which is inserted into plant cells contains a gene which encodes a selectable marker such as an antibiotic resistance marker, but this is not mandatory. An exemplary drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers et al., Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988).

Techniques for genetically engineering plant cells and/or tissue with an expression cassette comprising an inducible promoter or chimeric promoter fused to a heterologous coding sequence, including possibly an antisense DNA construct and/or a DNA construct designed to elicit double-stranded RNA-mediated gene silencing, followed by a transcription termination sequence are to be introduced into the plant cell or tissue by *Agrobacterium*- mediated transformation, electroporation, microinjection, particle bombardment or other techniques known to the art. The expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin.

A DNA construct carrying a plant-expressible gene or other DNA of interest can be inserted into the genome of a plant by any suitable method. Such methods may involve, for example, the use of liposomes, electroporation, diffusion, particle bombardment, microinjection, gene gun, chemicals that increase free DNA uptake, e.g., calcium phosphate coprecipitation, viral vectors, and other techniques practiced in the art. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, such as those disclosed by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 120,516 (Schilperoort et al.). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells.

The choice of vector in which the DNA of interest is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication, protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. The vector desirably includes a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition, preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells.

Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline, among other selective agents. The neomycin phosphotransferase gene has the advantage that it is expressed in eukaryotic as well as prokaryotic cells.

Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, CA) and pPL, pK and K223 available from Pharmacia (Piscataway, NJ), and pBLUESCRIPT and pBS available from Stratagene (La Jolla, CA). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al., eds., Cold Spring Harbor Press (1989) and the Lambda ZAP vectors available from Stratagene (La Jolla, CA). Other exemplary vectors include pCMU [Nilsson et al. (1989) *Cell* 58:707]. Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/Kb and pCMUII used in various applications herein are modifications of pCMUIV [Nilsson, (1989) supra].

Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells and capable of directing stable integration within the host plant cell include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers et al. (1987) *Meth. in Enzymol.* **153**:253-277, and several other expression vector systems known to function in plants. See for example, Verma et al., No. WO87/00551; Cocking and Davey (1987) *Science* **236**:1259-1262.

A transgenic plant can be produced by any means known to the art, including but not limited to *Agrobacterium tumefaciens*-mediated DNA transfer, preferably with a disarmed T-DNA vector, electroporation, direct DNA transfer, and particle bombardment [See Davey et al. (1989) *Plant Mol. Biol.* **13**:275; Walden and Schell (1990) *Eur. J. Biochem.* **192**:563; Joersbo and Burnstedt (1991) *Physiol. Plant.* **81**:256; Potrykus (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**:205; Gasser and Fraley (1989) *Science* 244:1293; Leemans (1993) *Bio/Technology* **11**:522; Beck et al. (1993) *Bio/Technology* **11**:1524; Koziel et al. (1993) *Bio/Technology* **11**:194; Vasil et al. (1993) *Bio/Technology* **11**:1533 and Gelvin, S.B. (1999) *Curr. Opin. Biotech.* **9**:227-232]. Techniques are well-known to the art for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues.

Many of the procedures useful for practicing the present invention, whether or not described herein in detail, are well known to those skilled in the art of plant molecular biology. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York, Kaufman (1987) in Genetic Engineering Principles and Methods, J.K. Setlow, ed., Plenum Press, NY, pp. 155-198; Fitchen et al. (1993) Annu. Rev. Microbiol. 47:739-764; Tolstoshev et al. (1993) in Genomic Research in Molecular Medicine and Virology, Academic Press; Ausubel, F.M. et al. (1997) "Current Protocols in Molecular Biology" Wiley, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

TABLE I

Specific pathogens for the major crops

<u>Soybeans</u>: *Phytophthora megasperma* fsp. *glycinia, Macrophomina phaseolina, Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium oxysporum, Diaporthe phaseolorum* var. *sojae (Phomopsis sojae), Diaporthe phaseolorum* var. *caulivora, Sclerotium rolfsii, Cercospora kikuchii, Cercospora sojina, Peronospora manshurica, Colletotrichum dematium (Colletotichum 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 ultimum, Pythium debaryanum,* Tomato spotted wilt virus, *Heterodera glycines Fusarium solani;* <u>Canola</u>: *Albugo candida, Alternaria brassicae, Leptosphaeria maculans, Rhizoctonia solani, Sclerotinia sclerotiorum, Mycosphaerella brassiccola, Pythium ultimum, Peronospora parasitica, Fusarium roseum, Alternaria alternata;* <u>Alfalfa</u>: *Clavibater michiganese subsp. insidiosum, 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 oxysporum, Rhizoctonia solani, Uromyces striatus, Colletotrichum trifolii* race 1 and race 2, *Leptosphaerulina briosiana, Stemphylium botryosum, Stagonospora meliloti, Sclerotinia trifoliorum,* Alfalfa Mosaic Virus, *Verticillium albo-atrum, Xanthomonas campestris* p.v. *alfalfae, Aphanomyces euteiches, Stemphylium herbarum, Stemphylium alfalfae;* <u>Wheat</u>: *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*, *Collotetrichum graminicola*, *Erysiphe graminis* f.sp. *tritici*, *Puccinia graminis* f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, *Puccinia striiformis*, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*, *Septoria avenae*, *Pseudocercosporella herpotrichoides*, *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris 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 arrhenomannes*, *Pythium gramicola*, *Pythium aphanidermatum*, High Plains Virus, European 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 carotovorum* pv. *carotovora*, *Cephalosporium acremonium*, *Phytophthora cryptogea*, *Albugo tragopogonis*; Corn: *Fusarium moniliforme* var. *subglutinans*, *Erwinia stewartii*, *Fusarium moniliforme*, *Gibberella zeae (Fusarium graminearum)*, *Stenocarpella maydi (Diplodia maydis)*, *Pythium irregulare*, *Pythium debaryanum*, *Pythium graminicola*, *Pythium splendens*, *Pythium 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*, *Kabatiella zeae*, *Colletotrichum graminicola*, *Cercospora zeae-maydis*, *Cercospora sorghi*, *Ustilago maydis*, *Puccinia sorghi*, *Puccinia polysora*, *Macrophomina phaseolina*, *Penicillium oxalicum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia pallescens*, *Clavibacter michiganense* subsp. *nebraskense*, *Trichoderma viride*, Maize Dwarf Mosaic Virus A & B, Wheat Streak Mosaic Virus, Maize Chlorotic Dwarf Virus, *Claviceps sorghi*, *Pseudonomas avenae*, *Erwinia chrysanthemi* pv. *zea*, *Erwinia corotovora*, *Cornstunt spiroplasma*, *Diplodia macrospora*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora maydis*, *Peronosclerospora sacchari*, *Spacelotheca reiliana*, *Physopella zeae*, *Cephalosporium maydis*, *Caphalosporium acremonium*, Maize Chlorotic Mottle Virus, High Plains Virus, Maize Mosaic Virus, Maize Rayado Fino Virus, Maize Streak Virus, Maize Stripe Virus, Maize Rough Dwarf Virus; <u>Sorghum</u>: *Exserohilum turcicum*, *Colletotrichum graminicola (Glomerella graminicola)*, *Cercospora sorghi*, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*, *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)*, *Sphacelotheca cruenta*, *Sporisorium sorghi*, Sugarcane mosaic H, Maize Dwarf Mosaic Virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthona macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*, etc.

CLAIMS:

1. A method for improving disease resistance in a plant by down-regulating, mutating or inactivating a cyclic nucleotide-gated ion channel (*CNGC*) *DND* gene or gene product.

2. The method of claim 1 wherein said disease is a result of a plant pathogen.

3. The method of claim 2 wherein said plant pathogen is selected from the group consisting of viruses, bacteria, and fungi.

4. The method of claim 3 wherein said pathogen is a virus selected from the nepovirus group including Tobacco ringspot virus.

5. The method of claim 3 wherein said pathogen is a gram-negative bacterium, including bacteria of the genus *Pseudomonas* or *Xanthorionas,* including *Pseudomonas syringae pv. tomato* and *Xanthomonas campestris pv. campestris.*

6. The method of claim 3 wherein said pathogen is an ascomycete funcus, including fungi of the genus *Erysiphe,* including *Eryshiphe orontii.*

7. The method of claim 1 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO: 2.

8. The method of claim 7 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under low stringency conditions.

9. The method of claim 7 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under medium stringency conditions.

10. The method of claim 7 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under high stringency conditions.

11. The method of claim 1 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO: 5.

12. The method of claim 11 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under low stringency conditions.

13. The method of claim 11 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under medium stringency conditions.

14. The method of claim 11 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under high stringency conditions.

15. The method of claim 1 wherein said down regulation or inactivation is achieved by expressing a *CNGC* or *DND* antisense or sense molecule in said plant.

16. The method of claim 15 wherein the *CNGC* or *DND* antisense or sense molecule is expressed under control of an inducible promoter.

17. The method of claim 16 wherein the inducible promoter is a pathogen-inducible promoter.

18. A transformed plant or plant tissue or seed modified according to the method of claim 1.

19. A transformed plant or plant tissue or seed comprising a *CNGC* or *DND* antisense molecule.

20. A transformed plant or plant tissue or seed comprising a *CNGC* or *DND* sense molecule.

21. A method for improving disease resistance in a plant by administering an inhibitor of *CNGC* activity into said plant.

22. A method for controlling cell death in a plant by down-regulating, mutating or inactivating a *CNGC* or *DND* gene or gene product.

23. The method of claim 22 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO:2.

24. The method of claim 23 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under low stringency conditions.

25. The method of claim 23 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under medium stringency conditions.

26. The method of claim 23 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under high stringency conditions.

27. The method of claim 22 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO: 5.

28. The method of claim 27 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under low stringency conditions.

29. The method of claim 27 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under medium stringency conditions.

30. The method of claim 27 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under high stringency conditions.

31. The method of claim 22 wherein said down regulation or inactivation is achieved by using a *CNGC* or *DND* antisense or sense molecule.

32. The method of claim 31 wherein the sense or antisense molecule is expressed under control of an inducible promoter.

33. The method of claim 32 wherein the inducible promoter is a pathogen-inducible promoter.

34. A method for reducing hypersensitive response in response to a pathogen attack in a plant by down-regulating, mutating or inactivating a cyclic nucleotide-gated ion channel *CNGC* or *DND* gene or gene product.

35. The method of claim 34 wherein said pathogen is selected from the group consisting of viruses, bacteria, and fungi.

36. The method of claim 35 wherein said pathogen is a virus selected from the nepovirus group consisting of Tobacco ringspot virus.

37. The method of claim 35 wherein said pathogen is a gram-negative bacterium, including bacteria of the genus *Pseudomonas* or *Xanthorionas,* including *Pseudomonas syringae pv. tomato* and *Xanthomonas campestris pv. campestris.*

38. The method of claim 35 wherein said pathogen is an ascomycete funcus, including fungi of the genus *Erysiphe,* including *Eryshiphe orontii.*

39. The method of claim 34 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO: 2.

40. The method of claim 39 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under low stringency conditions.

41. The method of claim 39 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under medium stringency conditions.

42. The method of claim 39 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 2 under high stringency conditions.

43. The method of claim 34 wherein said *CNGC* or *DND* gene is homologous to SEQ ID NO: 5.

44. The method of claim 43 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under low stringency conditions.

45. The method of claim 43 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under medium stringency conditions.

46. The method of claim 43 wherein said *CNGC* or *DND* gene is one hybridizing with SEQ ID NO: 5 under high stringency conditions.

47. The method of claim 34 wherein said down regulation or inactivation is achieved by expressing a *CNGC* or *DND* antisense or sense molecule in said plant.

48. The method of claim 47 wherein the sense or antisense molecule is expressed under control of an inducible promoter.

49. The method of claim 47 wherein the inducible promoter is a pathogen-inducible promoter.

50. A method for identifying a disease resistance gene in a plant by screening for a *CNGC* or *DND* gene.

51. The method of claim 50 wherein said *CNGC* or *DND* gene is *AtCNGC2/DND1* as given in SEQ ID NO:2.

52. The method of claim 50 wherein said *CNGC* or *DND* gene is *AtCNGC1/DND2* as given in SEQ ID NO: 5.

FIG. 1A

Col / Psg

FIG. 1B

Col / Psg (*avrRpt2+*)

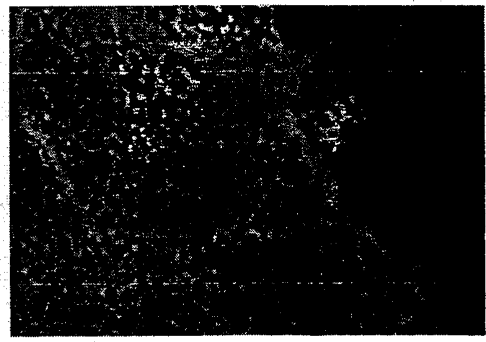


FIG. 1C

dnd1 / Psg

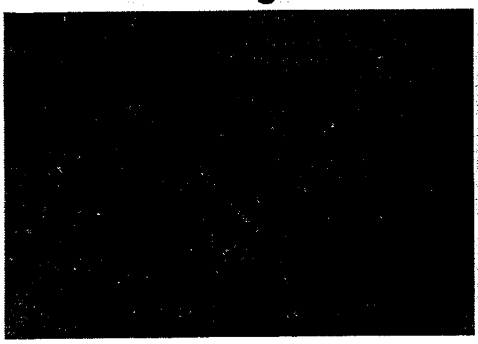


FIG. 1D

dnd1 / Psg (*avrRpt2+*)

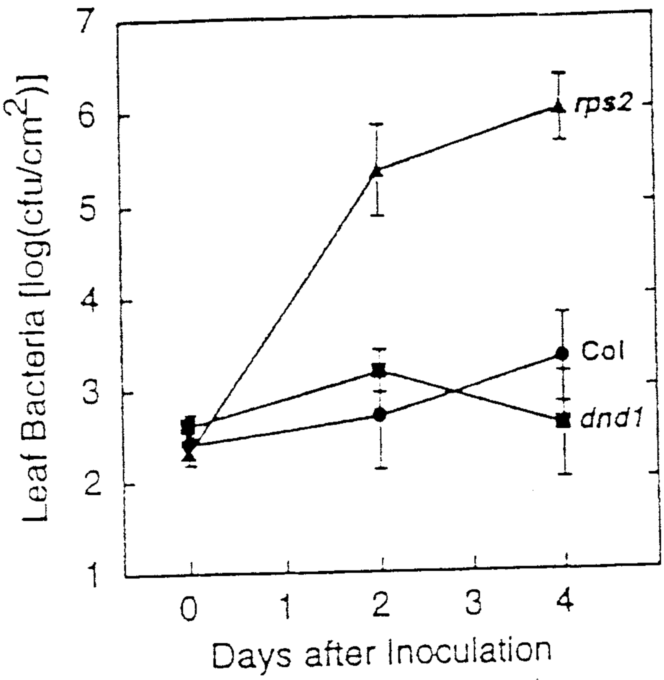

Leaf Bacteria [log(cfu/cm2)]
7
6
5
4
3
2
1
rps2
Col
dnd1
0
1
2
3
4
Days after Inoculation

FIG. 2A

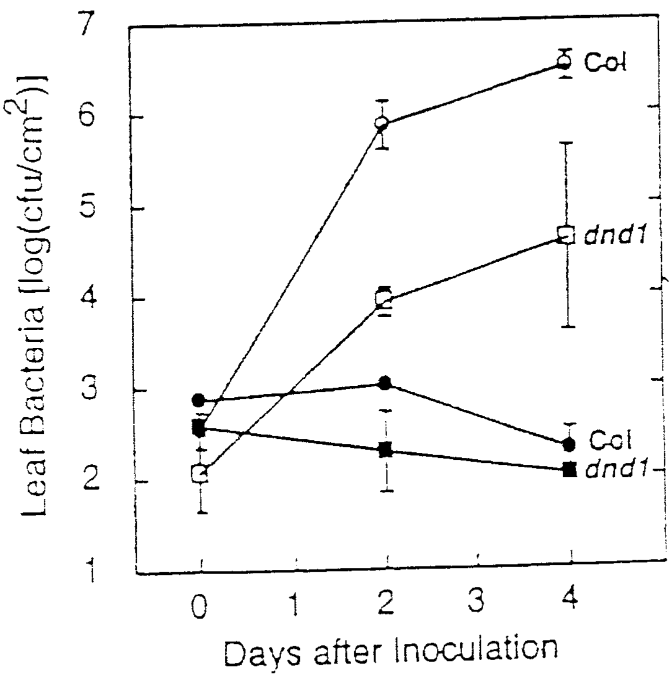

Leaf Bacteria [log(cfu/cm2)]
7
6
5
4
3
2
1
Col
dnd1
Col
dnd1
0
1
2
3
4
Days after Inoculation

FIG. 2B

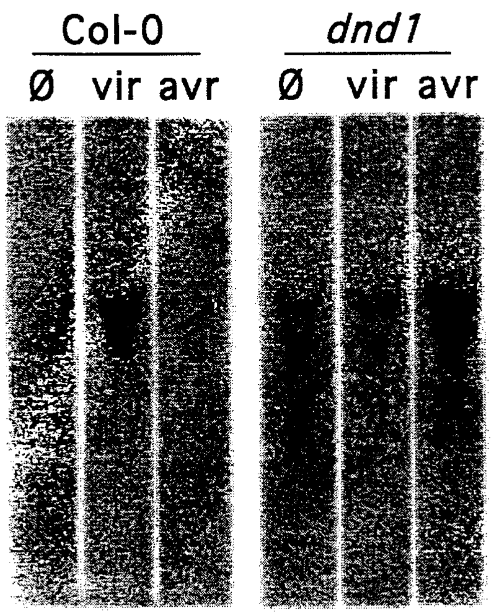


FIG. 3A

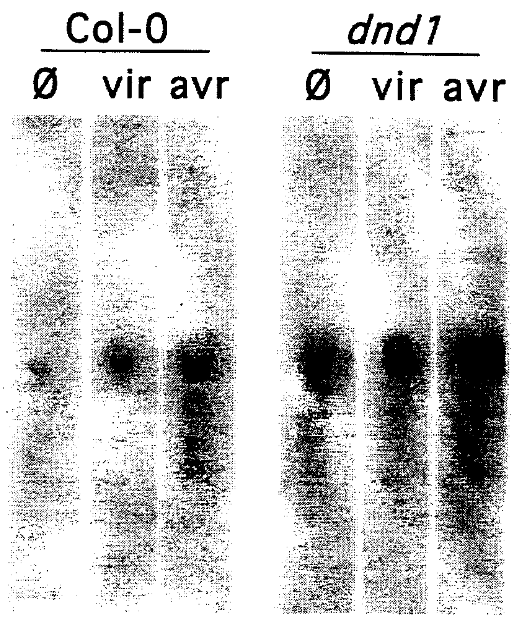


FIG. 3B

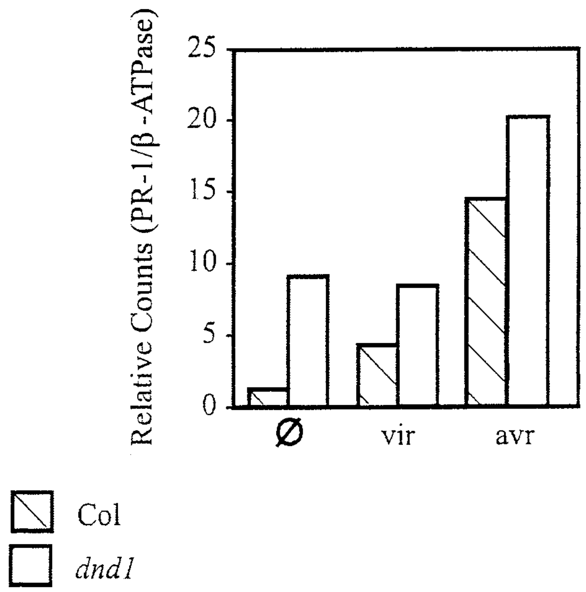


FIG. 3C

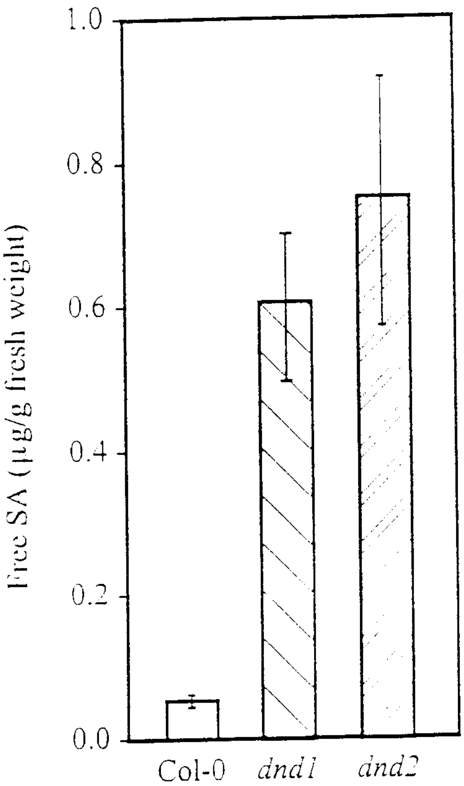

1.0
0.8
0.6
0.4
0.2
0.0
Free SA (μg/g fresh weight)
Col-0
dnd1
dnd2

FIG. 4A

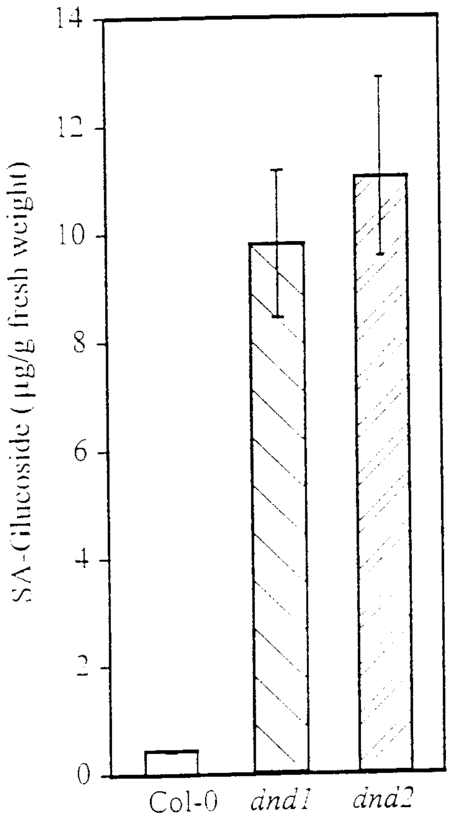
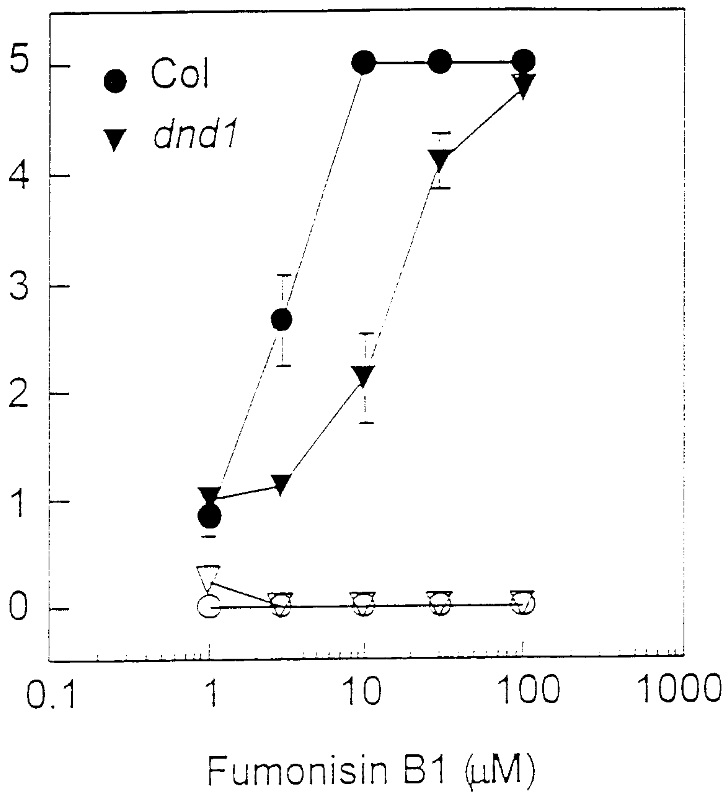
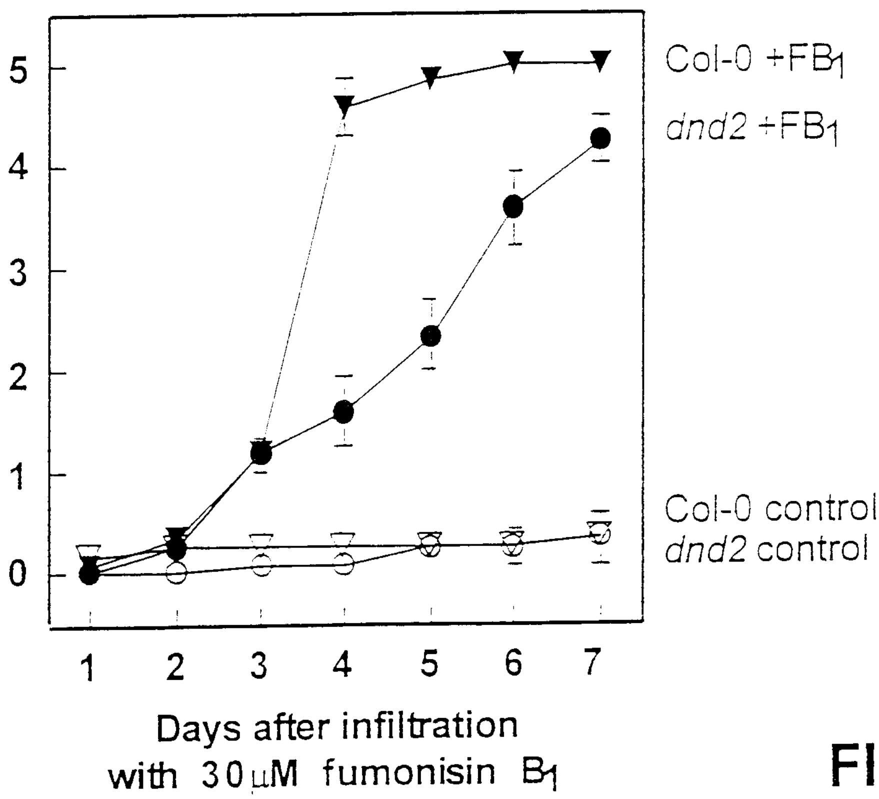

14
12
10
8
6
4
2
0
SA-Glucoside (μg/g fresh weight)
Col-0
dnd1
dnd2

FIG. 4B



5
4
3
2
1
0
Col
dnd1
0.1
1
10
100
1000
Fumonisin B1 (µM)

FIG. 5A



5
4
3
2
1
0
Col-0 +FB1
dnd2 +FB1
Col-0 control
dnd2 control
1
2
3
4
5
6
7
Days after infiltration
with 30µM fumonisin B1

FIG. 5B

6/20

1	GAATTCGCCT	TGCGTACCCT	ACTGGCGGAC	GGGTATAAAT
41	AAGGAATAGA	AAGTCTCGGG	AAAAAGGGGA	CGTTTTTCGG
81	GACCTGGAGA	GAGGATAGCA	CAAGTCAGCT	CGCCCAGATC
121	ATCCAGTTCG	CCCAGAAGAG	CCAGCTCATC	GATAGAAGAC
161	AGCTCGTCGA	GAGAAGTCGC	CTCGTCAAGA	TCATCCAGCT
201	CGCCCAGAAG	AGTCAGCTCA	TCGATACAAG	TCGGCTCGTC
241	GAGAGAAGTC	CGCTCATCGA	AGATCATCCA	GCTCGCCCAG
281	AAGAGCCAGC	TCACCAGAAC	CACCAGCTCG	AAACAGCATC
321	GCCTCGTAGC	GCCCTCAACT	ATGCTCTCAG	TTCGCAACAA
361	GCCTTTCAGC	CCGTCGATTG	TCCGGCTTCA	AGTTCAGCTC
401	GTGGATTAGA	GATTGATTCT	TCACCTTCCC	CAGAGACTCA
441	CCCGTACTTG	TTTAATTCGT	GCATTAACAG	TCCTCTACTA
481	CATGCAATAT	TAGAATGAGT	TTGAACAAAG	TTATTCATCT
521	ACCATACCCA	TTTATTCATC	TGCACTGTAT	CCTTAGTTAT
561	TTATTCCAAT	AAACGCTGAA	TTTTATCCAA	CCCAAAAATA
601	AATAAATAGA	AATTTGTGTT	CGATGTGTGT	GGTCCGCTGA
641	TATTGGTCGT	CACTCGAATT	ATTAATGCAT	GATATGGATT
681	TGTATGACTG	ACAAATTTTT	GGAAACTTCA	CTGTCAGGTA
721	CGTGTGTACT	ATATATGACC	ACAAAAGTGC	ATTTTATCAT
761	ATATATTAGT	AAACTAACTG	TAGGTAATTT	TTTTATTTTA
801	AAAAGTGTAA	GCAATAATGA	AAATTGAAAA	AATGAGAGTA
841	TTATGCTGCA	AAAAAACATT	ATAGTAACTG	GAGTATCTCT
881	GTATCTGCAA	TCAACAAAGG	CTTAATTACG	AGATTAGGCT
921	GGTCGTATGT	CTCCGTTGTT	ATATCATAGG	GATATGGCGT
961	ACGTCAACAT	GTATTCAATC	CGTAATAAAA	ACACATATAT
1001	AACAAGTATC	AATTGACGGC	AAACAAAATA	TAGTATATAA
1041	CAAACATATA	TATCAATAAT	TGAGTCAAAT	ACATCTATCA
1081	GTAAATGTTA	ATGACAACGA	CAAAAATCAT	TATCATGAAA
1121	ATCTGTTATT	TGTATAAATA	ATGAATGTTT	GACAAAAAAA
1161	AGTATAATTA	ATGAATAATA	TCAAATGATA	ATATGCTTAA
1201	ATCGTACATT	CACTAAAATA	TCTTCTACTG	TATTTATAGA
1241	GTTAGAATTA	TGACATCTTC	CTATATAAAG	CAAGTAAATT
1281	TGTAAACTTA	TCTAAAAGTT	CAATGATTAA	ACGTAAAGAA
1321	ATTACATTTA	AAATGATGGG	TCCATTTTAA	CGTAGCAATC
1361	CTCATGATCT	GTACGGAATC	CAATTTGTTC	AATGTCTATT
1401	TGTGGGCCGT	TTTGCTAAGC	AAGCCACCAC	ATCTCTCAGA
1441	CACTTGACAG	CTCATCATCT	CCCCCTTGTC	AATCCCGGTT
1481	CGGTTTCTGA	TTTAGTTCGA	TCTTCGATTT	TAGTTATTAA
1521	ACCGGAATCT	CCCCTAACAA	ATAAACCGAA	TCCTACTAAG
1561	AGACGTACGT	ATCACACACA	AATCTTGCGG	CTTTGCCTCT
1601	CTATACAAAT	CACTCTTCTC	TCTCCCAATC	ACTCCCTGCA
1641	AATTTTCTTC	TCTCCCTCTC	CCATGGTGGT	TCCTCTATTT
1681	CAATCATGCC	CTCTCACCCC	AACTTCATCT	TCAGGTTTTT
1721	ATACCACACA	ATTCCCATTT	TTTTTCATAT	CTATATTCGT
1761	TCATTAATGG	TGCTTTCACG	CTAAAACACT	CCCGAATTCT
1801	GAATATGTAT	TTTGTGTCTT	TTGCTGTTTA	CCCAGAGGTT
1841	ACAAGCTTTA	CCTGAGATTG	ATTATTTTTC	CCCGAGAAAC
1881	ACTTTTCCCG	GAAAATTCAC	TCGTTTTTTG	AACTGTTCTA
1921	TAGCTAACAA	GTCACCATGC	AATTCCTTAT	AATCTCGCTT
1961	GAGTATGCGA	AATCGTTCAT	CTAGCAACGA	AATCGATTTT

FIG. 6/1

2001 CATAATTGTG TACTATATAA CGAATGTGAG ATGATTCTGA
2041 ATGATTTTTG GCGATAGATT GCTAATTCCT GATTACAATT
2081 TGAATATTTC AGGTGGATTG GACTGTTTTC CGATAAGTTC
2121 CGTCGACAAA CGACTGGGAT CGATGAAAAC AGTAACCTCC
2161 AAATCAACGG TGGAGATTCG AGCAGCAGCG GCAGCGATGA
2201 GACGCCGGTG CTAAGCTCCG TCGAGTGTTA CGCTTGCACA
2241 CAAGTAGGCG TCCCAGCTTT CCATTCAACT AGCTGCGATC
2281 AAGCTCACGC GCCGGAGTGG CGTGCCTCCG CCGGCTCTTC
2321 TCTAGTTCCG ATCCAGGAAG GATCTGTCCC TAACCCAGCC
2361 CGAACCAGAT TCCGACGTCT CAAAGGTCCG TTTGGTGAAG
2401 TTCTCGATCC TAGGAGCAAG CGCGTGCAGA GATGGAACCG
2441 CGCGTTGCTT TTAGCTCGTG GGATGGCTTT AGCGGTGGAT
2481 CCGCTCTTCT TCTACGCGCT TTCCATCGGC CGAACTACCG
2521 GACCGGCGTG TCTTTACATG GATGGTGCGT TCGCCGCGGT
2561 GGTCACGGTG CTCCGCACGT GTCTCGATGC TGTTCATCTT
2601 TGGCACGTGT GGCTTCAATT CAGACTGGCC TACGTCTCGA
2641 GAGAGTCGCT TGTCGTTGGT TGTGGGAAGC TCGTTTGGGA
2681 TCCACGCGCC ATCGCGTCTC ACTACGCACG CTCTCTCACT
2721 GGCTTCTGGT TTGATGTTAT CGTCATCCTC CCTGTCCCTC
2761 AGGTGAATTT TCAGAACAAC CTTCCAACTA TTCCAATATT
2801 ATGAAATTAA CCCTAAACTT TTTATTACTA TCAATAAAGC
2841 CCATATTTAC ATTTGAAAGT GTTACCTGTT GATGAATCAT
2881 GATTTGAGAT GCAGATAAGA TGTGAACTTT GTGTTTTTGC
2921 TTGTTTGCAG GCAGTGTTTT GGTTAGTTGT GCCGAAACTG
2961 ATAAGAGAAG AGAAGGTTAA GCTGATAATG ACGATTCTGC
3001 TGCTAATATT CTTGTTCCAG TTCCTCCCCA AGATTTATCA
3041 CTGCATCTGT TTGATGAGAA GGATGCAGAA GGTCACTGGT
3081 TACATTTTTG GAACTATTTG GTGGGGTTTT GCTCTTAATC
3121 TCATCGCATA TTTCATCGCT TCTCATGTAA GTCCTCTCAA
3161 GCTAGATATT GTATTTCTGC CTGAATATAA TGTCCTGCAA
3201 CAATAGGTTA TATTCAGGTC CTAAAACAAA TGCAATATAA
3241 CAAATCCCAT GTGTATATAT ATAGTACTAC TAAGGTCTGT
3281 CCTAAGATAT GAAATTAAAG TTTGTTGTTT ATGGAAATTC
3321 TGGTGGTTTC AGGTTGCTGG GGGATGTTGG TATGTTCTCG
3361 CAATACAGCG TGTTGCTTCT TGCATAAGAC AACAATGTAT
3401 GAGAACCGGG AACTGCAATC TGAGTCTGGC TTGCAAAGAA
3441 GAGGTCTGTT ACCAATTTGT GTCACCGACA AGCACAGTTG
3481 GATATCCATG CTTATCTGGA AACCTTACCA GTGTGGTCAA
3521 TAAGCCTATG TGCTTAGACT CTAACGGACC ATTCCGATAT
3561 GGTATCTACC GTTGGGCACT TCCAGTCATC TCCAGCAACT
3601 CTCTTGCGGT TAAGATCCTT TACCCCATCT TCTGGGGCCT
3641 AATGACTCTC AGGTAATTGC TTTGTTTCTG AGCTTAAGGT
3681 TTACATCTTG GATATAAAAA AATTCTCTGA TTGACATACT
3721 GAATCTGTTT TGGGGGTCGC GTTTTACAGC ACATTTGCGA
3761 ATGATCTTGA GCCCACAAGC AACTGGCTCG AGGTTATTTT
3801 CAGTATAGTT ATGGTTCTAA GTGGCTTGTT ACTTTTCACG
3841 CTGTTGATAG GAAACATTCA GGTAAACTAT GCAAGAATAT
3881 GTCTTTTCTA TGATATTGTG TTTTCATGGA ATGTAAGATT
3921 TTAAAAAGCT TTTTACTTTA CAATATTTTG AAGGTGTTTT
3961 TGCATGCGGT AATGGCGAAA AAAAGGAAAA TGCAGATACG
4001 GTGTAGGGAT ATGGAATGGT GGATGAAACG TAGGCAGTTA

FIG. 6/2

4041 CCTTCCCGGT TAAGACAGAG GGTTAGGCGA TTTGAGCGGC
4081 AGAGATGGAA TGCCTTGGGT GGTGAAGACG AGCTAGAACT
4121 TATACATGAT TTGCCTCCGG GTCTTCGAAG AGATATCAAA
4161 CGATATCTTT GCTTTGATCT CATTAACAAG GTACTAGGAA
4201 GCAGACTTTA TACAATCTTG TTAAGACTTG TGAAAGCAGT
4241 AAGTGTTAAG TCTCTGACAT GTTTGATCTT TCTCAGGTGC
4281 CATTGTTCAG GGGCATGGAC GACTTGATCC TCGACAACAT
4321 TTGCGATCGG GCTAAGCCTC GAGTCTTCTC TAAAGACGAA
4361 AAGGTACTCA TCCCATCATT TCTAAAAATT TATATTTCCA
4401 ATATTTTGCT TCGGTTTCGG TTTAAGAGTT TACTTGAAGA
4441 GTAAAATCTG AGGTTCTTCG ATTTTGTTGA TTGTCACAGA
4481 TCATCCGTGA AGGAGATCCT GTACAGAGAA TGATATTCAT
4521 CATGCGTGGA CGAGTCAAAC GTATACAGAG CCTAAGCAAA
4561 GGCGTCCTAG CCACTAGTAC ACTAGAACCA GGCGGTTACT
4601 TGGGCGACGA GCTACTCTCA TGGTGCCTAC GTCGCCCGTT
4641 TCTGGACCGT CTTCCCCCTT CCTCAGCAAC ATTTGTCTGC
4681 CTAGAAAACA TCGAGGCATT CTCCCTCGGA TCCGAAGATC
4721 TTAGGTACAT TACCGATCAT TTCCGTTATA AATTCGCGAA
4761 CGAGCGGCTT AAGCGGACCG CAAGATACTA TTCCTCAAAC
4801 TGGAGGACGT GGGCAGCGGT AAATATTCAG ATGGCGTGGC
4841 GCCGGCGTAG GAAAAGAACC CGTGGTGAAA ACATCGGCGG
4881 TTCGATGAGT CCTGTGTCGG AGAATAGCAT TGAAGGTAAC
4921 AGTGAACGCC GGTTACTTCA GTATGCAGCT ATGTTCATGT
4961 CCATTCGACC GCATGATCAT CTCGAATAAT AATAAAAAGT
5001 TCCAATTTTA TATCGGTCCA AATAATTGTG TGTTCATTGT
5041 TCCTCCTCAA TTGTTTACCT TTACTTCTTT AAAAAATCTT
5081 TCTTTGGAAT GATACTTCAA CCTCTTACGG TTAAGTTCAA
5121 AGTAGATTTT TTGAGCAACA AAATCTTGGA GGAGTTTATC
5161 CTTGGTAATT TTTTATTTTT TATTTTTTAT TAGCAGTAAA
5201 AGTAATTGTC TTAATAAGTA TAAACGACGT CGTTAGTAAG
5241 TACGACGTTT GCATATATCC TATATAGACT TTTTGTCCAA
5281 GCTCAGGACT TGCTAAAGCT CGTTCAGAGA CTCCAAAAGA
5321 GTTCATAAAC AAAAACCTAC TCCCAGAAAG TTCTTCCAAA
5361 TCTCTCGCCA TGGTTTCTCT CTTCTTTCGT GAATTTTAGT
5401 TTTTCTAAAT TTAATTATAC TTTAAATTGC TAAGTCCAAA
5441 CTCTTTCGCT TTTGATTCAT CGGTAATACA AGTTTAAGAA
5481 AAAAAATTCT TATATTTATC TCTCAAATCG AGTTTCGAGT
5521 GTTTGATTTG ATTCGTAAAA TCAATGGCGA CGAGCAAACC
5561 TCAAAGATCG CCGGCTGAAA TTGAGGATAT AATCCTCCGG
5601 AAGATATTTT ATGTAACCCT AACGGAATCA ACTGATTCCG
5641 ATCCTCGTAT TGTTTACTTG GAGATGACGG CCGCTGAGAT
5681 TCTCAGCGAA GGTAAAGAGC TTTTGCTTTC TCGTGATTTG
5721 ATGGAGAGAG TTCTCATAGA TCGTCTCTCC GGCGACTTTT
5761 CCGACGCCGA ACCGCCTTTC CCGTATTTAA TCGGTTGCCA
5801 CCGTCGTGCT TACGACGAAT CGAAGAAAAT CCAGTCGATG
5841 AAGGATAAGA ATCTGAGATC GGAGATGGAG ATTGTCACTA
5881 AACAAGCGAA GAAGCTT

FIG. 6/3

MPSHPNFIFRWIGLFSDKFRRQTTGIDENSNLQINGGDSSSSGS

DETPVLSSVECYACTQVGVPAFHSTSCDQAHAPEWRASAGSSLVPIQEGSVPNPARTR

FRRLKGPFGEVLDPRSKRVQRWNRALLLARGMALAVDPLFFYALSIGRTTGPACLYMD

GAFAAVVTVLRTCLDAVHLWHVWLQFRLAYVSRESLVVGCGKLVWDPRAIASHYARSL

TGFWFDVIVILPVPQAVFWLVVPKLIREEKVKLIMTILLLIFLFQFLPKIYHCICLMR

RMQKVTGYIFGTIWWGFALNLIAYFIASHVAGGCWYVLAIQRVASCIRQQCMRTGNCN

LSLACKEEVCYQFVSPTSTVGYPCLSGNLTSVVNKPMCLDSNGPFRYGIYRWALPVIS

SNSLAVKILYPIFWGLMTLSTFANDLEPTSNWLEVIFSIVMVLSGLLLFTLLIGNIQV

FLHAVMAKKRKMQIRCRDMEWWMKRRQLPSRLRQRVRRFERQRWNALGGEDELELIHD

LPPGLRRDIKRYLCFDLINKVPLFRGMDDLILDNICDRAKPRVFSKDEKIIREGDPVQ

RMIFIMRGRVKRIQSLSKGVLATSTLEPGGYLGDELLSWCLRRPFLDRLPPSSATFVC

LENIEAFSLGSEDLRYITDHFRYKFANERLKRTARYYSSNWRTWAAVNIQMAWRRRRK

RTRGENIGGSMSPVSENSIEGNSERRLLQYAAMFMSIRPHDHLE

FIG. 7

```
   1 ctccctgcaa atttcttctc tccctctccc atggtggttc ctctatttca atcatgccct
  61 ctcaccccaa cttcatcttc aggtggattg gactgttttc cgataagttc cgtcgacaaa
 121 cgactgggat cgatgaaaac agtaacctcc aaatcaacgg tggagattcg agcagcagcg
 181 gcagcgatga gacgccggtg ctaagctccg tcgagtgtta cgcttgcaca caagtaggcg
 241 tcccagcttt ccattcaact agctgcgatc aagctcacgc gccggagtgg cgtgcctccg
 301 ccggctcttc tctagttccg atccaggaag gatctgtccc taacccagcc cgaaccagat
 361 tccgacgtct caaaggtccg tttggtgaag ttctcgatcc taggagcaag cgcgtgcaga
 421 gatggaaccg cgcgttgctt ttagctcgtg ggatggcttt agcggtggat ccgctcttct
 481 tctacgcgct ttccatcggc cgaactaccg gaccggcgtg tctttacatg gatggtgcgt
 541 tcgccgcggt ggtcacggtg ctccgcacgt gtctcgatgc tgttcatctt tggcacgtgt
 601 ggcttcaatt cagactggcc tacgtctcga gagagtcgct tgtcgttggt tgtgggaagc
 661 tcgtttggga tccacgcgcc atcgcgtctc actacgcacg ctctctcact ggcttctggt
 721 ttgatgttat cgtcatcctc cctgtccctc aggcagtgtt ttggttagtt gtgccgaaac
 781 tgataagaga agagaaggtt aagctgataa tgacgattct gctgctaata ttcttgttcc
 841 agttcctccc caagatttat cactgcatct gtttgatgag aaggatgcag aaggtcactg
 901 gttacatttt tggaactatt tggtggggtt ttgctcttaa tctcatcgca tatttcatcg
 961 cttctcatgt tgctgggggga tgttggtatg ttctcgcaat acagcgtgtt gcttcttgca
1021 taagacaaca atgtatgaga accgggaact gcaatctgag tctggcttgc aaagaagagg
1081 tctgttacca atttgtgtca ccgacaagca cagttggata tccatgctta tctggaaacc
1141 ttaccagtgt ggtcaataag cctatgtgct tagactctaa cggaccattc cgatatggta
1201 tctaccgttg ggcacttcca gtcatctcca gcaactctct tgcggttaag atcctttacc
1261 ccatcttctg gggcctaatg actctcagca catttgcgaa tgatcttgag cccacaagca
1321 actggctcga ggttattttc agtatagtta tggttctaag tggcttgtta cttttcacgc
1381 tgttgatagg aaacattcag gtgtttttgc atgcggtaat ggcgaaaaaa aggaaaatgc
1441 agatacggtg tagggatatg gaatggtgga tgaaacgtag gcagttacct tcccggttaa
1501 gacagagggt taggcgattt gagcggcaga gatggaatgc cttgggtggt gaagacgagc
1561 tagaacttat acatgatttg cctccgggtc ttcgaagaga tatcaaacga tatctttgct
1621 ttgatctcat taacaaggtg ccattgttca ggggcatgga cgacttgatc ctcgacaaca
1681 tttgcgatcg ggctaagcct cgagtcttct ctaaagacga aaagatcatc cgtgaaggag
1741 atcctgtaca gagaatgata ttcatcatgc gtggacgagt caaacgtata cagagcctaa
1801 gcaaaggcgt cctagccact agtacactag aaccaggcgg ttacttgggc gacgagctac
1861 tctcatggtg cctacgtcgc ccgtttctgg accgtcttcc ccctttcctca gcaacatttg
1921 tctgcctaga aaacatcgag gcattctccc tcggatccga agatcttagg tacattaccg
1981 atcatttccg ttataaattc gcgaacgagc ggcttaagcg gaccgcaaga tactattcct
2041 caaactggag gacgtgggca gcggtaaata ttcagatggc gtggcgccgg cgtaggaaaa
2101 gaacccgtgg tgaaaacatc ggcggttcga tgagtcctgt gtcggagaat agcattgaag
2161 gtaacagtga acgccggtta cttcagtatg cagctatgtt catgtccatt cgaccgcatg
2221 atcatctcga ataataataa aaagttccaa ttttatatcg gtccaaataa ttgtgtgttc
2281 attgttcctc ctcaattgtt tacctttact tctttaaaaa atcttttcttg gaatgatact
2341 tcaacctctt acggttaagt tcaaagtaga tttttgagc aacaaaatct tggaggag
```

FIG. 8

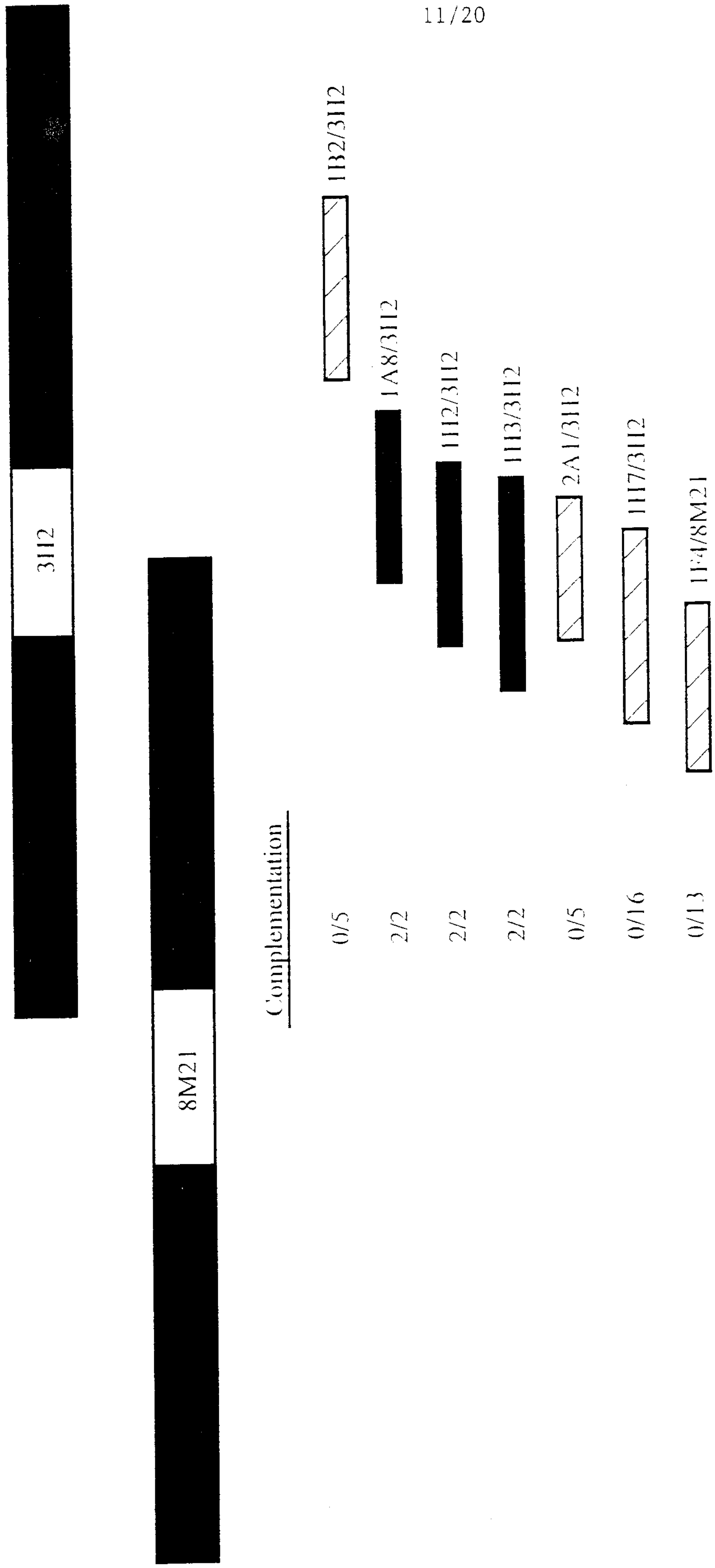

3H2
8M21
Complementation
1B2/3H2
0/5
1A8/3H2
2/2
1H2/3H2
2/2
1H3/3H2
2/2
2A1/3H2
0/5
1H7/3H2
0/16
1F4/8M21
0/13

FIG. 9

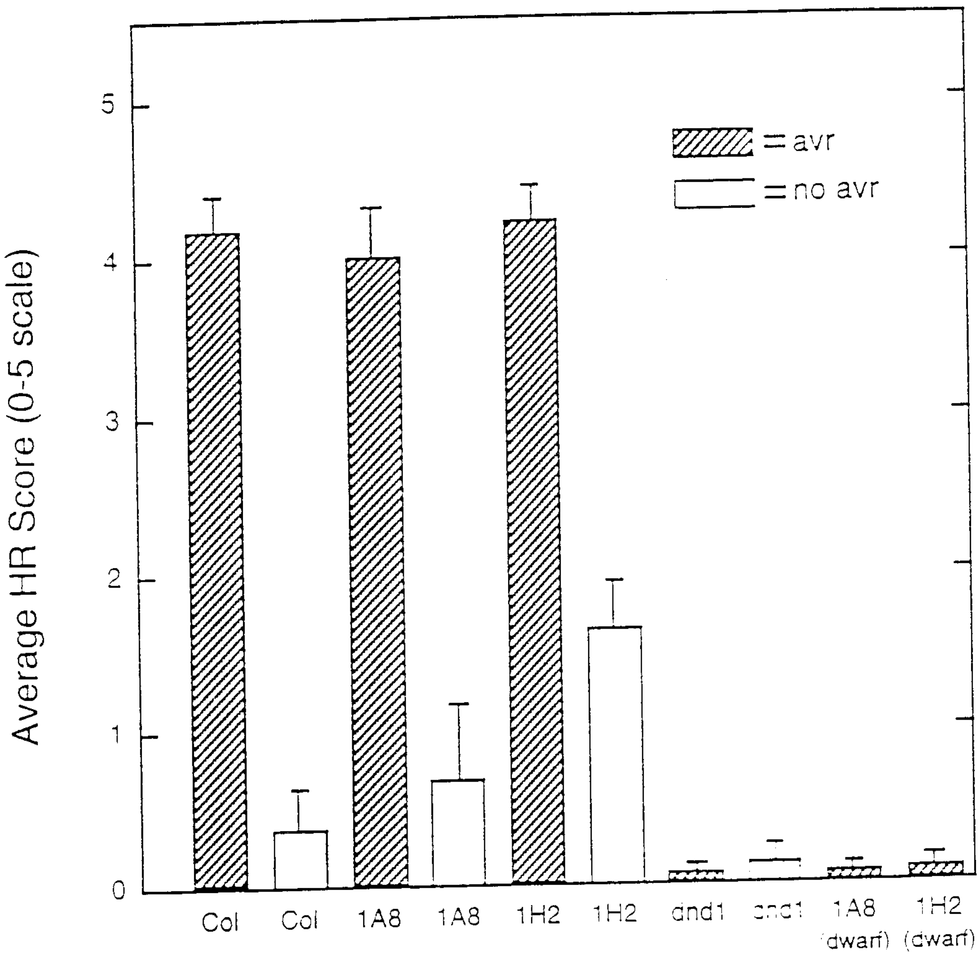

Average HR Score (0-5 scale)
0
1
2
3
4
5
= avr
= no avr
Col
Col
1A8
1A8
1H2
1H2
dnd1
1A8 (dwarf)
1H2 (dwarf)

FIG. 10

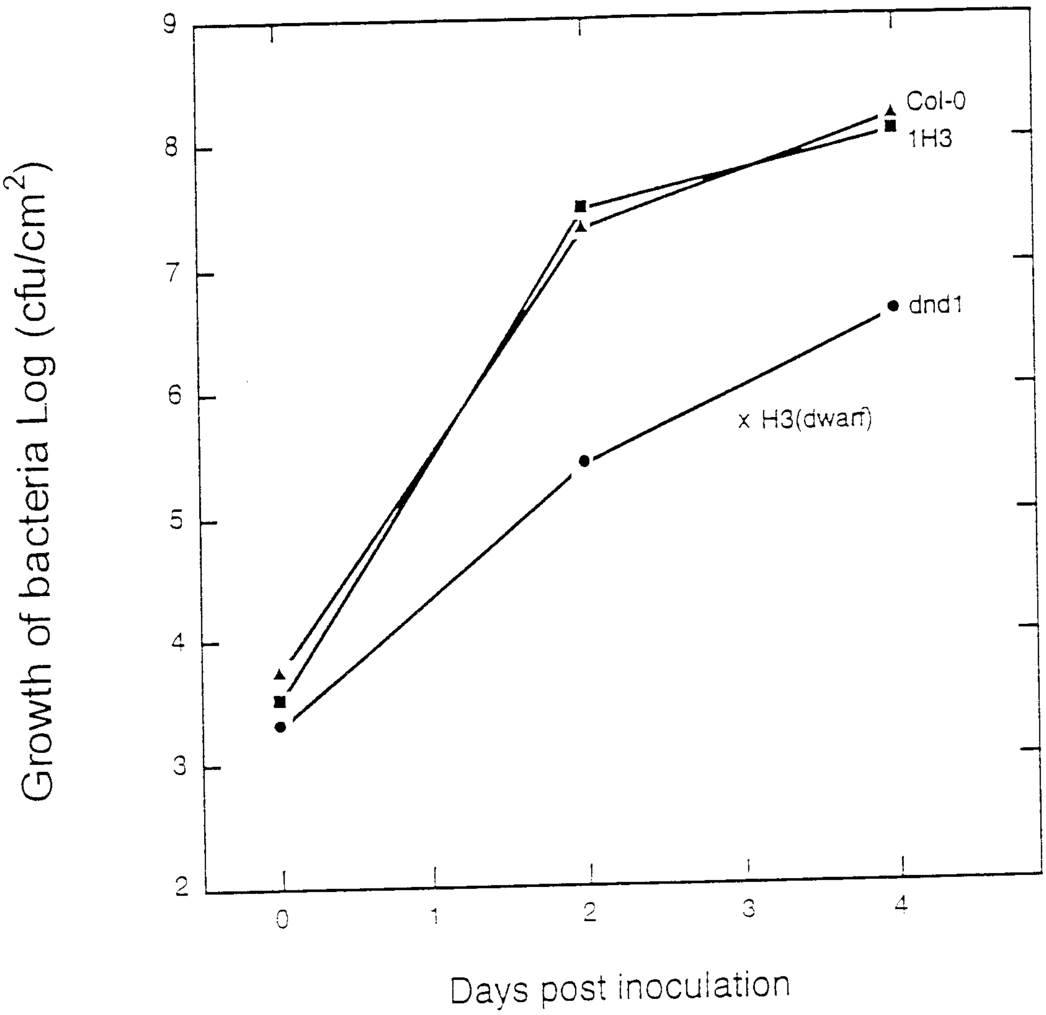

Growth of bacteria Log (cfu/cm²)
9
8
7
6
5
4
3
2
0
1
2
3
4
Days post inoculation
Col-0
1H3
dnd1
x H3(dwarf)

FIG. 11

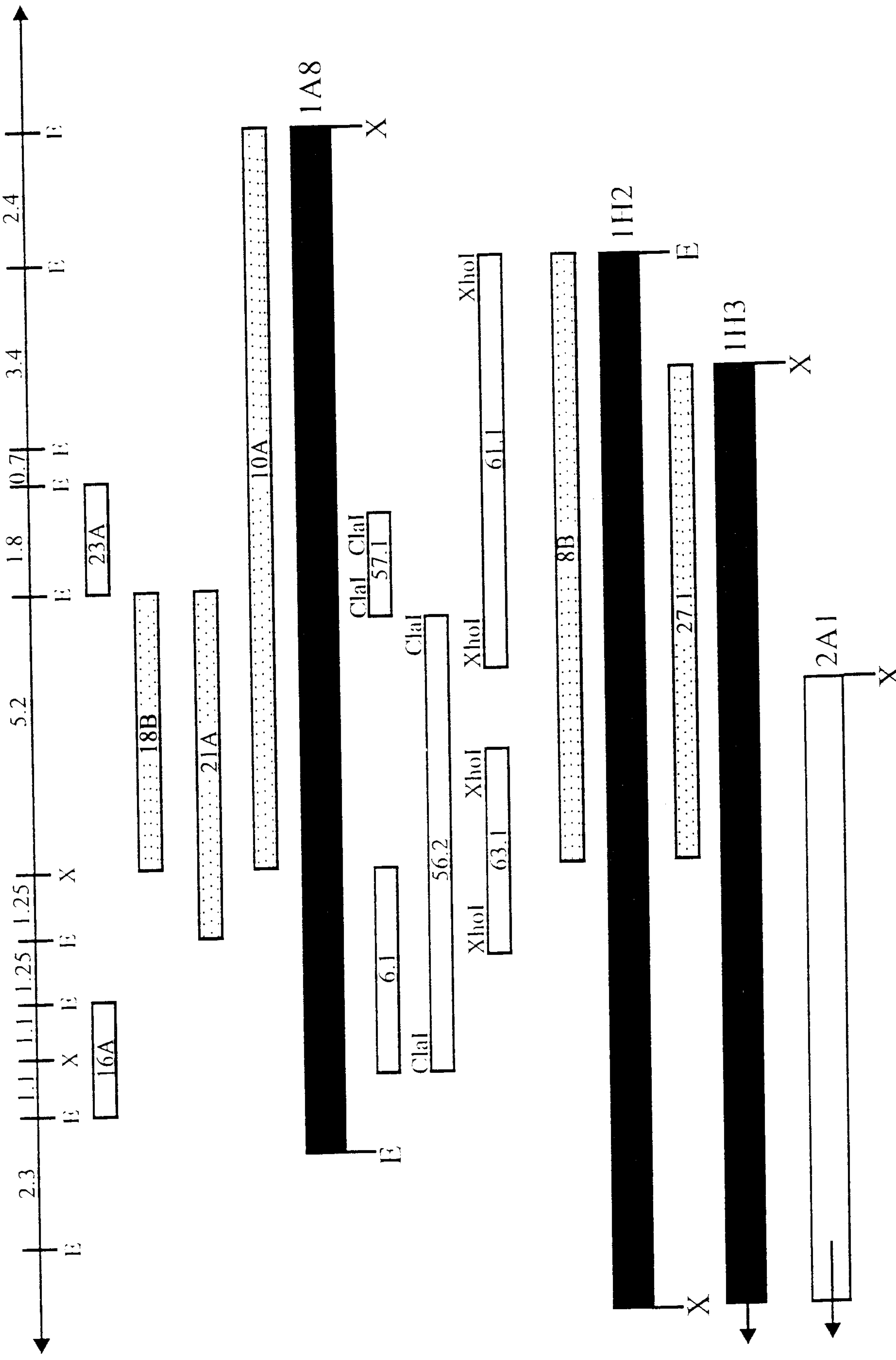

2.4
3.4
0.7
1.8
5.2
1.25
1.25
1.1
1.1
2.3
E
X
23A
16A
18B
21A
10A
1A8
ClaI
57.1
6.1
56.2
XhoI
61.1
63.1
8B
1H2
27.1
1H3
2A1

FIG. 12

ATGCACTGTGCCTGTGTGACTAATGGGTACTCTAGTGCCATTTGGAAGTG
AGACTGTAATACCAGTAACAGGAAAAGTCTCACTAAACATTGCCAAATC
AGAACAAACATGACTACTAGCTCCACTGTCAACTATCCAAGCATCATGA
GGCAAAATGGAATATAATGATGAAAGACAATGATGTTGAAATGTGAAAG
AATGATTTTCATATCGTAAACTTGTTGAGGGAAATTCAATAGAAACATTA
CCAGCTGTAGAAGTAGTTGCCATAGCTCCATGATCAGTGATGGTAGCTGA
TGGAAAGGGAGTTGACTGCTCCGGAACATGAAGATGAGTATTGAGTTGTT
GTATTAAAGAATGGACTTGATCCGTCGTAAAAAGACTGAGATCAAGACT
TGATGCAGTCATTGCAGGAGGTGGAATATAAGGAGTAGATCCCATGACT
GCATTAGCAACAGCATTATGTCATTTTTCATTCAAATCCGAAGAAGAGAA
AGATACTCTTACAAATGTCTTTTTCTTGTGATAATTTTACGGTGGGAAGA
TGGTTGACTTTGACTCATATCGTCCATATTCAAAGGTCTTGATAGTTTGAA
TTCGATGTTAATAGTATTTTATTCGTATAAACAATTAAATAAGAGTTGACA
ATGTTTAGACAAATTTGGATTATGAAGTAGTAACAATGATAAAATCTTGA
GACTGGTCTCCAAATCCGACAGCTAGGAACCTCCCTTGGCATTCATAAGA
CAACGTACAATCAGGGACACGATACGGTCAACGGTCTCTATTAGCACTT
AATTATCAGTATCAATTAAACTATCAATTGAGTAATATAATAGACGTTCA
AATTTAATTTTAAACTTAACGGTCTAAATTTGTGGAGAACAAAATTTGGT
AAAAATGAGAAGATAAAGAGGAAGAGGACACGTGGGTTGGTTCGTGGTT
GATCGACAAGTACTCACGGGCTATATCTTTAACTTATCATCCTTTTATTCT
TAACAAAAGAAATTTGGTGGAGATAAATTTAATCATGGTCATGGTTTTAG
TAGCTACAAATGCAAATGTGATTTTCTTTTTTCGTCTGCGTTTGCAAGTTTG
AAATTTTTGGTGCTGATGAAAAAAAGAAGAGATGATGTTTCACCATTTTT
TTTTTTGTTTTTTGATCAAAGGATGTTTCACCAACATAAGAAAAGAAAAC
ATGTCATGAAGGAGTTAGGACTAACAGTTTGAAAATACTTAAGAACTCA
AATGTTAAAAAGGCTCATATTTTTTTTCCCAAATTATGTCATCTCCTTCTAT
AATCTAAATCATTCGTGATTTAACATAAAACTAAATATTAAGTAACTTTA
TTCAAATCTATGAAATCGATTTACCATATACACGATTTTAAAAAGGATAC
AAGCGGAAATGAAACTGTGCATTGAAATATACGAACAAAAAGCCTGAGT
TTACGAAATTTGACATTATCTCATCAACACGAAACTTCGACGTATTACAA
AGACAAAAATCAAACCTACAAAAATAGAGAACAATAGTCAGTTACAGA
ATTTAAGTTTGCCACGAAAATGATACATCATTATGAAGTTTTTGTATGCCT
AAACATTGATATCAGTTAATGTCTTCGACTGTAATAAAGAAATGCCTTGA
TTAACTCCGTAACTACGTCTTCTTTTGTCAGCAAATTCTAATTTATTACATG
CTTAAGTTGATTTGATCCCATAACCAAACAACATATATATGGAATGTATA
TAAAAAAAATCTAAGCAATCTAAAAAAATTAATTATAATATATGCTATA
AAAAAAATTATAGGAAACTTTGCTAAATGGCATAAACAGTTATTTTAATT
AAGTATATGGAATTGTCAAAAAATTATCATTATTTTTTTAAAAATACATG
ATTGACTCTTTTTTTTTGTGTGCAAACAAATACATGATTGACAAAAAAAA

FIG. 13/1

..ATACATGATTGATAAAGATATTTCTTTCAACATCTATGGATGAGAGGAT
CAGGAAATGTAGCATTATTTTACTTTTGAAAACTACATATATTATTGTTGT
GAGCCTGTGAATTCTGTAATCTTCCAACATATTCGTCTACTAAAAAACTG
ACAACTCAATTCAGAACATATTTTTCTACTAAAAGGTGAAGTTAAAAACT
CAATTTAAAAAAGATTTCGTACTTAATAGTCAAAGTTAAATTTGGTCCGA
AATCTTTTTGAAGTATCAAAGACTGCAAAGGTATAACTGTCTTTTTGGAAT
AACTTTAAAAAAATTAACAAAAACAGTGAAACAAAATTTAAGAAAATC
GAAGAAAAAAATGATAAAATAGCAAAAGTTTGTGACTTTCGTGGAAACT
CCTTCCCCGTCCTCTCTGTCCTCTGATAAAGGCTTCACTTTCATCATTCAAT
CTCTTCTTCTTCTTTCTTCTTCATCACTCTCTCTCTTTCTCCAATTTCTTCATT
TTATCAGCTTCCTCTCTTCTTTAGTTTCTGGGTCACTTTAGTTTCGCTCTTTC
CGAGGAAAAACAAAACAAAGATTTGATTTTTCTTTTTGGTGGGTTTAAAC
AGTGATTTCTTCTTTTGCCCTTTTATTGGTTCTTATTGTTCTCCTCAAAAGTC
TACAGGATTGTCGGAAGCAAACACACTTGTCCATGTTCGGTTTGATTTTTT
ACCTCAAACAAGTTTCTTCCTTTTTTTTTGCTGTTTAGTCGAATTGCGTTTTT
GACACTGGAAAGTTTTGAAATTTGAGTTTGATTTTTACTGAAATCGAAATT
GTTGAATTGAATTGAAGTTTGTTGAATCAGGGTTATGAATTTCCCTCTAAT
TGGTGAAGAAGACACATGAAGCAGTGAAATCTCTGTTTGTATTGAATCTT
ATTAGTCTCAAACTATGAATTTCCGACAAGAGAAGTTTGTAAGGTCAGTG
TTCCAGATTTGTCTCATTGAATTCTAAGTCGTGAAGCTTAATTCGATTCTT
CTTCACTTTCTCGGATCAGGTTTCAAGATTGGAAGTCGGATAAGACTTCCT
CCGACGTGGAATATTCCGGTAAAAACGAGATTCAAACTGGAATATTCCA
GAGAACAATAAGCTCAATCTCCGACAAGTTTTACAGAAGCTTTGAATCA
AGCTCTGCAAGGATCAAACTATTCAAAAGATCTTACAAGTCTTACTCTTT
TAAAGAAGCTGTTTCAAAAGGGATTGGTTCTACTCACAAAATTCTTGACC
CACAAGGACCTTTTCTTCAGAGATGGAACAAGATCTTTGTTTTAGCTTGTA
TCATCGCTGTCTCGCTTGACCCTTTGTTCTTCTACGTGCCTATCATCGATGA
TGCTAAGAAATGTCTTGGTATTGACAAGAAAATGGAAATAACAGCAAGC
GTTTTGCGCTCTTTCACTGATGTTTTTTATGTCCTTCACATCATTTTCCAGTT
CCGTACTGGCTTTATCGCTCCTTCGTCTCGTGTTTTTGGGAGAGGTGTTCTT
GTTGAGGACAAGCGAGAGATCGCTAAACGTTACTTGTCCTCACATTTCAT
AATTGACATTCTTGCTGTTCTTCCGCTTCCGCAGGTGATTCATTGATTGGTT
TTGTTTGCATTTCCAAATCATTAGCTTGATTCTTTGAGTAATGTGAGTAATT
GTGTTTTTCCTCAGATGGTGATTTTGATAATCATTCCACATATGAGAGGTT
CATCGTCTTTGAACACGAAGAATATGTTGAAGTTTATTGTTTTCTTCCAAT
ATATACCGAGGTTTATAAGAATATATCCGCTCTACAAGGAAGTTACAAG
AACTTCAGGCATACTCACTGAGACAGCTTGGGCTGGAGCTGCTTTCAATC
TCTTCCTCTACATGCTTGCTAGTCATGTGAGTGCCAAATTGGTTCTAACTG
TCCCAACCTTTTTAGTTTCAGAGTTTAGATATATAATATTTTTGGTCAATA
CCTGTAAATGTGTAGGTGTTTGGTGCTTTCTGGTATTTGTTCTCAATCGAA
CGCGAAACGGTGTGCTGGAAACAAGCTTGTGAGAGGAATAACCCTCCGT
GCATTTCGAAGTTGTTGTACTGTGACCCTGAAACTGCAGGAGGCAATGCT
TTCCTCAATGAGTCTTGTCCGATTCAGACACCAAACACAACACTCTTCGA

FIG. 13/2

CTTTGGGATATTCCTTGACGCACTTCAATCCGGTGTAGTGGAATCTCAAG
ATTTCCCTCAAAAGTTTTTTTACTGTTTCTGGTGGGGTCTGCAGAACCTCA
GGTATTGAGAATTTATCAGCAAAATTTTCTCCGTTTCTTATGAAGAAAAGT
TTCAACACTAAGTTCTTGCATCTTTGTTCTGTTTTCGTGATCCAGTTCGCTC
GGTCAGAACCTTAAAACAAGTACATATATTTGGGAAATCTGTTTCGCGGT
GTTCATTTCTATTGCGGGGCTGGTTTTGTTCTCCTTCTTGATTGGAAATATG
CAGGTTAGTCTCATTTCATCTAATATGCCATAGTGTTTGCGAAATCGATTA
CTTACTGCTTTTCTTTTAAATGGCAGACGTATCTGCAATCCACTACCACGA
GATTGGAGGAGATGAGGGTAAAGAGAAGAGACGCAGAACAATGGATGT
CACACCGTTTGCTACCTGAGAACTTGAGAAAAAGAATCCGGCGATACGA
GCAGTACAAATGGCAAGAGACAAGAGGTGTTGACGAAGAGAATCTTCTT
AGTAATCTTCCCAAAGATCTTAGACGCGACATCAAACGTCATCTCTGTCT
CGCCCTTCTCATGCGGGTAAAGAAGAAATCATCTCCTCTCTTTACTCGATT
TCTCATTTATTGTCAACATCACTCGTAACCGCATCTTGTTTTTGTTGGTTCT
AGGTCCCCATGTTTGAGAAAATGGACGAACAGCTTCTTGATGCGCTCTGT
GACCGTTTGCAACCTGTGTTATACACAGAGGAAAGCTACATAGTAAGAG
AAGGAGATCCGGTAGACGAAATGCTCTTCATAATGCGCGGGAAGCTTCT
AACAATCACAACAAACGGTGGAAGAACCGGTTTTTTAAATTCCGAGTAT
CTTGGAGCCGGTGATTTCTGTGGTGAGGAGCTTTTAACCTGGGCTTTAGAC
CCACACTCATCCTCAAACCTCCCAATCTCAACAAGAACTGTTCGAGCTCT
CATGGAAGTTGAAGCTTTCGCACTTAAAGCTGATGACCTCAAATTCGTGG
CTTCCCAGTTCAGACGTCTTCACAGCAAACAGCTAAGACATACTTTCAGG
TACTACTCACAACAATGGAAGACTTGGGCCGCTTGCTTCATACAAGCCGC
TTGGAGAAGATACATTAAGAAGAAACTCGAAGAGTCTCTTAAAGAAGAA
GAGAATCGGTTGCAGGATGCTTTGGCTAAAGAAGCTTGTGGAAGTTCCCC
AAGCCTCGGTGCTACAATATACGCATCACGGTTTGCTGCAAATATCTTGC
GCACAATACGTAGGAGCGGATCAGTAAGGAAACCAAGGATGCCGGAAC
GAATGCCACCTATGCTACTTCAGAAACCAGCAGAGCCAGATTTCAACAG
TGATGATTAATTAATTAGCATATATGTATATGAAAGATTTTATTGCGAACC
ATAGAGCCAATATATAAATATAAGTCTTTTTATAAAGGAGTAAACTTAGA
GATACAAAGCCTTCAATGATTTATTATTCCTCTTCAAGAGTTCTACAAAGT
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GTGAAGCTACTTCTCTCCGCAGGAATGTGGGCAAAGCAAATGCGGCTCT
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FIG. 13/3

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FIG. 14

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FIG. 15

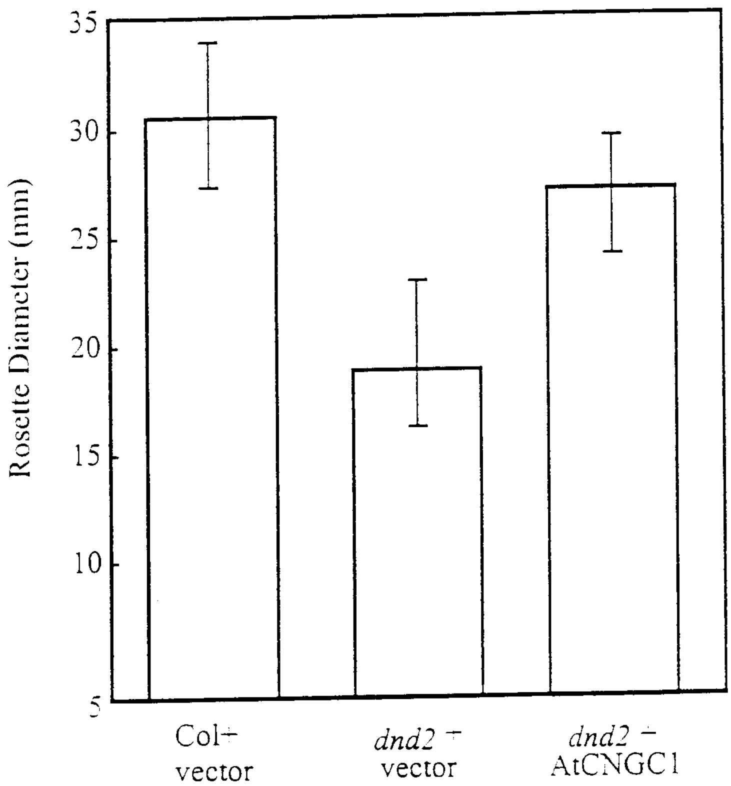

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FIG. 16

SEQUENCE LISTING

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Yu, I-Ching
Clough J., Steven
Fengler A., Kevin
Smith, Jr. K., Roger

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Val Leu Ser Ser Val Glu Cys Tyr Ala Cys Thr Gln Val Gly Val Pro
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Arg Glu Ser Leu Val Val Gly Cys Gly Lys Leu Val Trp Asp Pro Arg
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Glu Gly Asp Pro Val Gln Arg Met Ile Phe Ile Met Arg Gly Arg Val
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Lys Arg Ile Gln Ser Leu Ser Lys Gly Val Leu Ala Thr Ser Thr Leu
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675 680 685

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                20                  25                  30

caa act gga ata ttc cag aga aca ata agc tca atc tcc gac aag ttt   445
Gln Thr Gly Ile Phe Gln Arg Thr Ile Ser Ser Ile Ser Asp Lys Phe
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Tyr Arg Ser Phe Glu Ser Ser Ser Ala Arg Ile Lys Leu Phe Lys Arg
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Ser Thr His Lys Ile Leu Asp Pro Gln Gly Pro Phe Leu Gln Arg Trp
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Ile Phe Leu Asp Ala Leu Gln Ser Gly Val Val Glu Ser Gln Asp Phe
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Pro Gln Lys Phe Phe Tyr Cys Phe Trp Trp Gly Leu Gln Asn Leu Ser
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Ser Leu Gly Gln Asn Leu Lys Thr Ser Thr Tyr Ile Trp Glu Ile Cys
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ctt ccc aaa gat ctt aga cgc gac atc aaa cgt cat ctc tgt ctc gcc     1741
Leu Pro Lys Asp Leu Arg Arg Asp Ile Lys Arg His Leu Cys Leu Ala
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Leu Leu Met Arg Val Pro Met Phe Glu Lys Met Asp Glu Gln Leu Leu
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Arg Gly Lys Leu Leu Thr Ile Thr Thr Asn Gly Gly Arg Thr Gly Phe
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aaa cag cta aga cat act ttc agg tac tac tca caa caa tgg aag act    2173
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tgg gcc gct tgc ttc ata caa gcc gct tgg aga aga tac att aag aag    2221
Trp Ala Ala Cys Phe Ile Gln Ala Ala Trp Arg Arg Tyr Ile Lys Lys
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aaa ctc gaa gag tct ctt aaa gaa gaa gag aat cgg ttg cag gat gct    2269
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Tyr Ala Ser Arg Phe Ala Ala Asn Ile Leu Arg Thr Ile Arg Arg Ser
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gga tca gta agg aaa cca agg atg ccg gaa cga atg cca cct atg cta    2413
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Arg Ser Phe Glu Ser Ser Ser Ala Arg Ile Lys Leu Phe Lys Arg Ser
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Tyr Lys Ser Tyr Ser Phe Lys Glu Ala Val Ser Lys Gly Ile Gly Ser
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Thr His Lys Ile Leu Asp Pro Gln Gly Pro Phe Leu Gln Arg Trp Asn
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Lys Ile Phe Val Leu Ala Cys Ile Ile Ala Val Ser Leu Asp Pro Leu
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Phe Phe Tyr Val Pro Ile Ile Asp Asp Ala Lys Lys Cys Leu Gly Ile
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Asp Lys Lys Met Glu Ile Thr Ala Ser Val Leu Arg Ser Phe Thr Asp
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Ala Pro Ser Ser Arg Val Phe Gly Arg Gly Val Leu Val Glu Asp Lys
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Arg Glu Ile Ala Lys Arg Tyr Leu Ser Ser His Phe Ile Ile Asp Ile
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His Met Arg Gly Ser Ser Ser Leu Asn Thr Lys Asn Met Leu Lys Phe
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Ile Val Phe Phe Gln Tyr Ile Pro Arg Phe Ile Arg Ile Tyr Pro Leu
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Tyr Lys Glu Val Thr Arg Thr Ser Gly Ile Leu Thr Glu Thr Ala Trp
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Trp Lys Gln Ala Cys Glu Arg Asn Asn Pro Pro Cys Ile Ser Lys Leu
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Ser Cys Pro Ile Gln Thr Pro Asn Thr Thr Leu Phe Asp Phe Gly Ile
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Arg Thr Val Arg Ala Leu Met Glu Val Glu Ala Phe Ala Leu Lys Ala
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Asp Asp Leu Lys Phe Val Ala Ser Gln Phe Arg Arg Leu His Ser Lys
        595                 600                 605

Gln Leu Arg His Thr Phe Arg Tyr Tyr Ser Gln Gln Trp Lys Thr Trp
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Ala Ala Cys Phe Ile Gln Ala Ala Trp Arg Arg Tyr Ile Lys Lys Lys
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Ala Lys Glu Ala Cys Gly Ser Ser Pro Ser Leu Gly Ala Thr Ile Tyr
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Ala Ser Arg Phe Ala Ala Asn Ile Leu Arg Thr Ile Arg Arg Ser Gly
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/20216

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 15/05, 15/09, 15/29, 15/82; A01H 5/00, 5/10
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 468, 419; 536/23.1, 23.6, 24.1, 24.5; 800/278, 279, 286, 295, 298, 301

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	YU et al. Gene-for-gene Disease Resistance Without the Hypersensitive Response in Arabidopsis dnd1 Mutant. Proc. Natl. Acad. Sci. USA. June 1998, Vol. 95, pages 7819-7824, see pages 7819-7823.	1-5, 34-37 -------------- 6-33, 38-52
Y	US 5,792,904 A (RYALS et al) 11 August 1998 (11.08.98), see entire document, especially columns 2-3, 12-13, 16-18.	1-6, 15-22, 31-38, 47-50
Y, P	US 5,986,082 A (UKNES et al) 16 November 1999 (16.11.99) ,see entire document, especially columns 24-32.	1-6, 15-22, 31-38, 47-50
A	US 5,629,470 A (LAM et al) 13 May 1997 (13.05.97), see entire document.	1-52

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 02 NOVEMBER 2000	Date of mailing of the international search report 27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MEDINA A. IBRAHIM Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/US00/20216

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 468, 419; 536/23.1, 23.6, 24.1, 24.5; 800/278, 279, 286, 295, 298, 301

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN CAS, WEST2.0
terms: cyclic nucleotide-gated channel or CNGC, DND gene or gene product, Arabidopsis, cell death, HR, down-regulating, mutate, inactivate, pathogen or disease resistant