(12) STANDARD PATENT (11) Application No. **AU 2007291330 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Method for increasing resistance to pathogens in transgenic plants

(51) International Patent Classification(s)
A01H 5/00 (2006.01) ***C12N 15/82*** (2006.01)
C07K 14/415 (2006.01)

(21) Application No: **2007291330** (22) Date of Filing: **2007.08.22**

(87) WIPO No: **WO08/025711**

(30) Priority Data

(31) Number	(32) Date	(33) Country
06119815.6	**2006.08.30**	**EP**

(43) Publication Date: **2008.03.06**
(44) Accepted Journal Date: **2013.01.17**

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(56) Related Art
HALTERMAN, D. A. et al., The Plant Journal, April 2004, Vol. 38, No. 2, pages 215-226
SUN, X. et al., The Plant Journal, February 2004, Vol. 37, No. 4, pages 517-527
BIERI, S. et al., The Plant Cell, December 2004, Vol. 16, No. 12, pages 3480-3495

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
6. März 2008 (06.03.2008)

PCT

(10) Internationale Veröffentlichungsnummer
WO 2008/025711 A3

(51) Internationale Patentklassifikation:
C12N 15/82 (2006.01) *A01H 5/00* (2006.01)
C07K 14/415 (2006.01)

(21) Internationales Aktenzeichen: PCT/EP2007/058735

(22) Internationales Anmeldedatum:
22. August 2007 (22.08.2007)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
06119815.6 30. August 2006 (30.08.2006) EP

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(81) Bestimmungsstaaten *(soweit nicht anders angegeben, für jede verfügbare nationale Schutzrechtsart)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Bestimmungsstaaten *(soweit nicht anders angegeben, für jede verfügbare regionale Schutzrechtsart)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), eurasisches (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht:
— *mit internationalem Recherchenbericht*
— *vor Ablauf der für Änderungen der Ansprüche geltenden Frist; Veröffentlichung wird wiederholt, falls Änderungen eintreffen*
— *mit dem Sequenzprotokollteil der Beschreibung in elektronischer Form getrennt veröffentlicht; auf Antrag vom Internationalen Büro erhältlich*

(88) Veröffentlichungsdatum des internationalen Recherchenberichts: 24. April 2008

(54) **Title:** METHOD FOR INCREASING RESISTANCE TO PATHOGENS IN TRANSGENIC PLANTS

(54) **Bezeichnung:** VERFAHREN ZUR ERHÖHUNG DER PATHOGENRESISTENZ IN TRANSGENEN PFLANZEN

(57) **Abstract:** The present invention relates to a method for increasing the resistance to pathogens in transgenic plants and/or plant cells, where a DNA sequence which codes for a protein with a leucine-rich repeat (LRR) domain and/or with a kinase activity is introduced into the plant or plant cell and expressed therein. The present invention likewise relates to the use of nucleic acids which code for such a protein for producing transgenic plants or plant cells with an increased resistance to pathogens. The present invention further relates to a nucleic acid sequences which code for a protein which confers an increased resistance to pathogens in plants.

(57) **Zusammenfassung:** Die vorliegende Erfindung betrifft ein Verfahren zur Erhöhung der Pathogenresistenz in transgenen Pflanzen und/oder Pflanzenzellen, wobei eine DNA-Sequenz, die für ein Protein mit einer leucine-rich-repeat (LRR)-Domäne und/oder einer Kinaseaktivität kodiert, in die Pflanze bzw. Pflanzenzelle eingeführt und dort exprimiert wird. Die vorliegende Erfindung betrifft ebenfalls die Verwendung von Nukleinsäuren, die für ein solches Protein kodieren, zur Herstellung von transgenen Pflanzen bzw. Pflanzenzellen mit einer erhöhten Pathogenresistenz. Weiterhin betrifft die vorliegende Erfindung Nukleinsäuresequenzen, die für ein Protein kodieren, das eine erhöhte Pathogenresistenz in Pflanzen vermittelt.

WO 2008/025711 A3

Method for increasing resistance to pathogens in transgenic plants

The present invention relates to a method of increasing the pathogen resistance in transgenic plants and/or plant cells, where a DNA sequence which codes for a protein with a leucine-rich-repeat (LRR) domain and/or a kinase activity is introduced into the plant or plant cell and expressed therein. The present invention also relates to the use of nucleic acids which code for such a protein, for the generation of transgenic plants or plant cells with an increased pathogen resistance. The present invention furthermore relates to nucleic acid sequences which code for a protein which confers an increased pathogen resistance in plants.

Plant diseases which are caused by a variety of pathogens such as, for example, viruses, bacteria and fungi can lead to considerable yield losses in crop plant cultivation, which firstly has economic consequences, but also poses a risk to human nutrition. Chemical fungicides have been employed since last century for controlling fungal diseases. While the use of these substances has made it possible to reduce the extent of plant diseases, it cannot be ruled out even now that these compounds have a harmful effect on humans, animals and the environment. In order to reduce the use of traditional plant protection products to a minimum in the long term, it is therefore important to study the natural pathogen defense of a variety of plants against different pathogens, and to exploit them, in a targeted manner, by recombinant manipulation, for example by the introduction of external resistance genes or by the manipulation of the endogenous gene expression in plants, in order to generate pathogen-resistant plants.

Only a few approaches exist which impart a resistance to pathogens, especially fungal pathogens, to plants. This shortcoming can partly be attributed to the complexity of the biological systems in question. Another fact which stands in the way of obtaining resistances to pathogens is that little is known about the interactions between pathogen and plant. The large number of different pathogens, the infection mechanisms developed by these organisms

and the defense mechanisms developed by the plant phyla, families and species interact with one another in many different ways.

Fungal pathogens have developed essentially two infection strategies. Some fungi enter into the host tissue via the stomata (for example rusts, Septoria species, Fusarium species) and penetrate the mesophyll tissue, while others penetrate via the cuticles into the epidermal cells underneath (for example Blumeria species).

The infections caused by the fungal pathogens lead to the activation of the plant's defense mechanisms in the infected plants. Thus, it has been possible to demonstrate that defense reactions against epidermis-penetrating fungi frequently start with the formation of a penetration resistance (formation of papillae, strengthening of the cell wall with callose as the main constituent) underneath the fungal penetration hypha (Elliott et al. Mol Plant Microbe Interact. 15: 1069-77; 2002).

In many cases, however, the plant's defense mechanisms only confer an insufficient protection mechanism against the attack by pathogens.

The formation of a penetration resistance to pathogens whose infection mechanism comprises a penetration of the epidermal cells or of the mesophyll cells is of great importance both for monocotyledonous and for dicotyledonous plants. In contrast to other approaches concerning the mediation of resistance, it can probably make possible the development of a broad-spectrum resistance to obligate biotrophic, hemibiotrophic and necrotrophic fungi.

To date, quantitative resistance traits (resistance-QTLs) have frequently been introduced by hybridization in order to generate plants with resistance to fungi. However, the disadvantage of this method is that undesirable traits are frequently also introduced. Moreover, the breeding methods required for this purpose are very complicated and time-consuming.

Accordingly, it was an object of the present invention to provide a method of increasing the resistance of plants to penetrating pathogens.

The object is achieved by the embodiments characterized in the claims.

As a consequence, the present invention relates to a method of increasing the pathogen resistance in transgenic plants and/or plant cells, where a DNA sequence which codes for a protein which mediates an increased pathogen resistance, preferably an increased resistance to fungal pathogens, is introduced into the plant or plant cell and expressed therein.

In the context of a TIGS (=Transient Induced Gene Silencing) analysis in barley using the method of Schweizer et al. (2001), it has been found that, as the result of dsRNAi-mediated silencing of the RNR8 gene, the sensitivity of the plant to the fungal pathogen *Blumeria graminis* is increased, and that the RNR8 gene might therefore play a role in conferring the pathogen resistance of barley plants.

RNR8 belongs to the family of the leucine-rich-repeat (LRR)-comprising proteins with a kinase domain or a kinase activity. Members of this family play an important role in a variety of cellular processes such as the regulation of endosperm and pollen development (Li and Wurtzel (1998) Plant Mol. Biol. 37: 749-761; Muschietti et al. (1998) Plant Cell 10: 319-330), the regulation of meristem and floral development (Torii et al. (1996) Plant Cell 8: 735-746; Clark et al. (1997) Cell 89: 575-585; Kim et al. (2000) Plant Sci. 152: 17-26) and the gibberellin-induced fruit growth (van der Knapp et al. (1999) Plant Physiol. 120: 559-569). The LRR domain comprises 2 to 45 repeats of an amino acid sequence with 20 to 30 amino acids and generally folds into the shape of a horseshoe.

It has been demonstrated by a further member of this family, OsXa21 from rice, that it confers resistance to the bacterial pathogen *Xanthomonas oryzae* (Song et al. (1995) Science 270(5243): 1804-1806). With approximately 23%, the protein according to the invention only exhibits very weak sequence homology to OsXa21.

The proteins according to the invention confer an increase of the pathogen resistance, preferably of the resistance to fungal pathogens, in plants. They are preferably distinguished by the fact that they exhibit either a leucine-rich-repeat (LRR) domain or a kinase activity; especially preferably, the proteins according to the invention exhibit both traits. Without wishing to be bound to one theory, it is currently assumed that either the kinase activity or the LRR domain or both traits together are responsible for conferring the pathogen resistance. In what follows, the term "protein according to the invention" will be used for the sake of simplicity.

The nucleic acid sequence which, in the method of the present invention, is introduced into the plant or plant cell and codes for a protein according to the invention is selected from the group consisting of:

i) nucleic acid sequences comprising nucleotide sequences which correspond to the coding sequences of SEQ ID No. 1,

ii) nucleic acid sequences comprising nucleotide sequences which code for a protein with the amino acid sequence shown in SEQ ID No. 2,

iii) nucleic acid sequences comprising nucleotide sequences which have at least 60% sequence identity with the coding sequences of SEQ ID No. 1, and/or

iv) nucleic acid sequences comprising nucleotide sequences which, under stringent conditions, hybridize with a complementary strand of a nucleotide sequence of i) to iii).

"Resistance" means the prevention, the repression, the reduction or the weakening of disease symptoms of a plant which occur as the result of infection with a pathogen. The symptoms can be different in nature, but preferably comprise those which directly or indirectly lead to an adverse effect on the quality of the plant, the quantity of the yield, the suitability for use as feedingstuff or foodstuff, or else make sowing, growing, harvesting or processing of the harvested material more difficult.

In a preferred embodiment, the following disease symptoms are weakened, reduced or prevented: development of pustules and spore beds on the surfaces of the infected tissue, maceration of the tissue, spreading necroses of the tissue, accumulation of mycotoxins, for example from *Fusarium graminearum* or *F. culmorum*.

An "increased pathogen resistance" means that the defense mechanisms of a certain plant or in a part of a plant, for example in an organ, a tissue, a cell or an organelle, show, as a result of the application of the method according to the invention, increased resistance to one or more pathogens in comparison with a suitable control, for example the wild type of the plant ("control plant", "original plant"), to which the method according to the invention has not been applied, under otherwise identical conditions (such as, for example, climatic conditions, culture conditions, pathogen species and the like). It is preferred that, in a plant, at least the epidermis and/or the mesophyll tissue or the organs which have an epidermis and/or a mesophyll tissue, show an increased resistance to the pathogens. For example, the resistance in the leaves is increased.

In one embodiment, the resistance in the lemma, the palea and/or the glum (anther primordium).

The increased resistance manifests itself preferably in a reduced manifestation of the disease symptoms, where the disease symptoms - besides the abovementioned adverse effects - also comprise for example the penetration efficiency of a pathogen into the plant or plant cell or the proliferation efficiency of the pathogen in or on the same. In this context, the disease symptoms are reduced by preferably at least 10% or at least 20%, especially preferably by at least 40% or 60%, particularly preferably by at least 70% or 80%, most preferably by at least 90% or 95%, in comparison with the control plant.

For the purposes of the invention, "pathogen" means organisms whose interactions with a plant lead to the above-described disease symptoms; in particular, pathogens mean organisms from the kingdom Fungi. The pathogen is preferably a pathogen which penetrates the epidermis or the mesophyll cells, in particular, they are pathogens which enter plants via stomata and subsequently penetrate mesophyll cells. In this context, organisms which may be mentioned by preference are those of the Ascomycota and Basidiomycota phyla. Especially preferred in this context are the families Blumeriaceae, Pucciniaceae, Mycosphaerellaceae and Hypocreaceae.

Especially preferred organisms of these families are those which belong to the genera Blumeria, Puccinia, Fusarium or Mycosphaerella.

Very especially preferred are the species *Blumeria graminis*, *Puccinia triticina*, *Puccinia striiformis*, *Mycosphaerella graminicola*, *Stagonospora nodorum*, *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae* and *Microdochium nivale*.

In especially preferred embodiments, the method according to the invention leads to a resistance in

- barley against the pathogen *Puccinia graminis* f.sp. hordei (barley stem rust),
- in wheat against the pathogens *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium*

culmorum, *Puccinia graminis* f.sp. tritici (wheat stem rust), *Puccinia recondita* f.sp. tritici, *Puccinia striiformis*, *Septoria nodorum*, *Septoria tritici* and/or *Septoria avenae*,
- in maize against the pathogens *Fusarium moniliforme* var. subglutinans, *Puccinia sorghi* and/or *Puccinia polysora*,
- in sorghum against the pathogens *Puccinia purpurea*, *Fusarium moniliforme*, *Fusarium graminearum* and/or *Fusarium oxysporum*,
- in soybeans against the pathogens *Phakopsora pachyrhizi* and/or *Phakopsora meibromae*.

Another subject matter of the invention is an isolated nucleic acid molecule, comprising a nucleic acid sequence selected from the group consisting of:

i) nucleic acid sequences comprising nucleotide sequences which correspond to the coding sequences of SEQ ID No. 1,

ii) nucleic acid sequences comprising nucleotide sequences which code for a protein with the amino acid sequence shown in SEQ ID No. 2,

iii) nucleic acid sequences comprising nucleotide sequences which have at least 60% sequence identity with the coding sequences of SEQ ID No. 1, and/or

iv) nucleic acid sequences comprising nucleotide sequences which, under stringent conditions, hybridize with a complementary strand of a nucleotide sequence of i) to iii),

which codes for a protein which confers an increased pathogen resistance, preferably an increased resistance to fungal pathogens, in plants.

In a preferred embodiment, the term "nucleic acid (molecule)" as used in the present context additionally comprises the untranslated sequence located at the 3'- and at the 5'-terminus of the coding gene region: at least 500, preferably 200, especially preferably 100 nucleotides of the sequence upstream of the 5'-terminus of the coding region and at least 100, preferably 50, especially preferably 20 nucleotides of the sequence downstream of the 3'-terminus of the

coding gene region.

An "isolated" nucleic acid molecule is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably does not have any sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid originates (for example sequences located at the 5'- and 3'-termini of the nucleic acid). In various embodiments, the isolated molecule may comprise for example fewer than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid originates. All the nucleic acid molecules mentioned here may take the form of, for example, RNA, DNA or cDNA.

The nucleic acid molecules used in the method, for example a nucleic acid molecule with a nucleotide sequence of SEQ ID No. 1 or a part thereof, can be isolated using standard techniques of molecular biology and the sequence information provided herein. Also, it is possible to identify for example an homologous sequence, or homologous, conserved sequence regions, at the DNA or amino acid level using comparative algorithms as can be found for example on the NCBI homepage at http://www.ncbi.nlm.nih.gov. Essential parts of this sequence, or the entire homologous sequence, can be used as hybridization probe using standard hybridization techniques (such as, for example, described in Sambrook et al., vide supra) for isolating, from other organisms, further nucleic acid sequences which are useful in the method, by screening cDNA libraries and/or genomic libraries. Moreover, a nucleic acid molecule comprising a complete sequence as shown in SEQ ID No. 1 or a part thereof can be isolated by a polymerase chain reaction, where oligonucleotide primers based on the sequences stated herein or of parts thereof are used (for example, a nucleic acid molecule comprising the complete sequence or a part thereof can be isolated by a polymerase chain reaction using oligonucleotide primers which have been generated on the basis of the same sequence). For example mRNA can be isolated from cells (for example by the guanidinium thiocyanate extraction method by Chirgwin et al. (1979) Biochemistry 18: 5294 – 5299), and

cDNA can be generated therefrom by means of reverse transcriptase (for example Moloney MLV reverse transcriptase, obtainable from Gibco/BRL, Bethesda, MD or AMV reverse transcriptase, obtainable from Seikagaku Amerika, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for amplification by means of a polymerase chain reaction can be generated on the basis of the nucleic acid sequence shown in SEQ ID No. 1 or with the aid of the amino acid sequence shown in SEQ ID No. 2. A nucleic acid according to the invention can be amplified by means of standard PCR amplification techniques using cDNA or alternatively using genomic DNA as the template and suitable oligonucleotide primers. The nucleic acid thus amplified can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides which correspond to a nucleotide sequence which codes for a protein according to the invention can be generated by standard synthetic methods, for example using an automatic DNA synthesizer.

The term "sequence identity" between two nucleic acid sequences is understood as meaning the identity of the nucleic acid sequence over the entire sequence length in each case, in a preferred embodiment over the entire expressed sequence length, preferably cDNA, even more preferably over the coding sequence, preferably CDS, which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA; Altschul et al. (1997) Nucleic Acids Res. 25: 3389ff), with the following parameters being set:

Gap Weight: 50 Length Weight: 3

Average Match: 10 Average Mismatch: 0

For example, a sequence with at least 80% homology at the nucleic acid level with the sequence SEQ ID No. 1 will, upon comparison with the sequence of SEQ ID No. 1 using the above program algorithm with the above parameter set, have at least 80% homology.

In one embodiment, the present invention relates to nucleic acid sequences which have at least 60%, preferably at least 65, 70, 75 or 80%, especially preferably at least 82, 84, 86, 88 or 90% and most preferably at least 92, 94, 96, 98 or 99% sequence identity to the sequence shown in SEQ ID No. 1.

"Identity between two proteins" is understood as meaning the identity of the amino acids over a specific protein region, preferably over the entire protein length, in particular the identity which is calculated by comparison with the aid of software, for example the Lasergene Software from DNA Star Inc., Madison, Wisconsin (USA) using the CLUSTAL method (Higgins et al. (1989) Comput. Appl. Biosci. 5(2): 151). Homologies can also be calculated with the aid of the Lasergene software from DNA Star Inc., Madison, Wisconsin (USA) using the CLUSTAL method (Higgins et al. (1989) Comput. Appl. Biosci. 5(2): 151).

Preferably, "identity between two proteins" is understood as meaning the identity of the amino acid sequence over the entire sequence length in each case, which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA) the following parameters being set:

Gap Weight: 8 Length Weight: 2

Average Match: 2 912 Average Mismatch: 2 003

"Standard hybridization conditions" is to be understood in a broad sense and means, depending on the application, stringent or less stringent hybridization conditions. Such hybridization conditions are described, inter alia, in Sambrook and Russell, Molecular Cloning - A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, 2001) or

in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

The skilled worker would choose hybridization conditions which allow him to distinguish between specific and unspecific hybridizations.

For example, the conditions during the wash step can be selected from among low-stringency conditions (with approximately 2X SSC at 50°C) and high-stringency conditions (with approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3M sodium citrate, 3M NaCl, pH 7.0). In addition, the temperature may be raised during the wash step from low-stringency conditions at room temperature, i.e. approximately 22°C, up to higher-stringency conditions at approximately 65°C. Both parameters, salt concentration and temperature can be varied simultaneously or else individually, the other parameter in each case being kept constant. It is also possible to employ denaturing agents such as, for example, formamide or SDS during the hybridization. In the presence of 50% formamide, the hybridization is preferably carried out at 42°C. Some examples of conditions for hybridization and wash step are given hereinbelow:

(1) Hybridization conditions may be selected for example from among the following conditions:

a) 4X SSC at 65°C,

b) 6X SSC at 45°C,

c) 6X SSC, 100 µg/ml denatured, fragmented fish sperm DNA at 68°C,

d) 6X SSC, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA at 68°C,

e) 6X SSC, 0.5% SDS, 100 μg/ml denatured, fragmented salmon sperm DNA, 50% formamide at 42°C,

f) 50% formamide, 4X SSC at 42°C,

g) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrate at 42°C,

h) 2X or 4X SSC at 50°C (low-stringency condition),

i) 30 to 40% formamide, 2X or 4X SSC at 42°C (low-stringency condition),

j) 500 mM sodium phosphate buffer pH 7.2, 7% SDS (g/V), 1 mM EDTA, 10 μg/ml single stranded DNA, 0.5% BSA (g/V) (Church and Gilbert (1984) Proc. Natl. Acad.Sci. U.S.A. 81(7): 1991-1995.)

(2) Wash steps can be selected for example from among the following conditions:

a) 0.015M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C,

b) 0.1X SSC at 65°C,

c) 0.1X SSC, 0.5% SDS at 68°C,

d) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C,

e) 0.2X SSC, 0.1% SDS at 42°C,

f) 2X SSC at 65°C (low-stringency condition).

In one embodiment, the hybridization conditions are selected as follows:

A hybridization buffer comprising formamide, NaCl and PEG 6000 is chosen. The presence of formamide in the hybridization buffer destabilizes double-stranded nucleic acid molecules, which makes it possible to lower the hybridization temperature to 42°C without thereby reducing stringency. The use of salt in the hybridization buffer increases the renaturation rate of a Duplex, or the hybridization efficiency. Although PEG increases the viscosity of the solution, which has an adverse effect on renaturation rates, the presence of the polymer in the solution increases the concentration of the probe in the remaining medium, which enhances the hybridization rate. The composition of the buffer is as follows:

Hybridization buffer
250 mM sodium phosphate buffer pH 7.2
1 mM EDTA
7% SDS (g/v)
250 mM NaCl
10 µg/ml ssDNA
5% Polyethylene glycol (PEG) 6000
40% Formamide

The hybridizations are carried out overnight at 42°C. The following morning, the filters are washed 3x with 2×SSC + 0.1% SDS for approximately 10 min in each case.

Nucleic acid sequences which deviate from the nucleic acid sequence shown in SEQ ID No. 1

can be generated for example by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID No. 1 so that proteins are generated into which one or more amino acid substitutions, additions or deletions have been introduced in comparison with the sequence shown in SEQ ID No. 2. Mutations can be introduced into the sequence of SEQ ID No. 1 by means of standard techniques, such as, for example, site-specific mutagenesis and PCR-mediated mutagenesis. It is preferred to generate conservative amino acid substitutions on one or more of the predicted nonessential amino acid residues, that is to say on amino acid residues which have no effect on the kinase activity and/or on the LRR domain. In a "conservative amino acid substitution", an amino acid residue is exchanged for an amino acid residue with a similar side chain. Families of amino acid residues with similar side chains have been defined in the art. These families comprise amino acids with basic side chains (for example lysine, arginine, histidine), acidic side chains (for example aspartic acid and glutamic acid), uncharged polar side chains (for example glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), unpolar side chains (for example alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (for example threonine, valine, isoleucine) and aromatic side chains (for example tyrosine, phenylalanine, tryptophan). A predicted nonessential amino acid residue in the protein used in accordance with the invention is thus preferably exchanged for another amino acid residue from the same side-chain family. As an alternative, it is possible, in another embodiment, to introduce the mutations randomly over the entire sequence, or part of the sequence, which codes for the protein according to the invention, for example to screen for their ability of conferring pathogen resistance.

The term "DNA fragments" as used in the present context is understood as meaning DNA portions which code for a protein according to the invention, whose biological activity consists in that it confers an increase of the pathogen resistance. The DNA fragments preferably code for a protein with a kinase activity or with an LRR domain, especially preferably with both traits, where the proteins encoded by the DNA portions have essentially

the same kinase activity and/or the same LRR domain as the proteins encoded by the complete DNA sequence and where the increase according to the invention of the pathogen resistance can be achieved in transgenic plants using these fragments.

The term "protein fragments" as used in the present context is understood as meaning protein portions whose biological activity consists in that it confers an increase of the pathogen resistance (preferably the resistance of fungal pathogens) in plants. The protein fragments preferably have a kinase activity or an LRR domain, especially preferably both traits, where the protein portions have essentially the same kinase activity and/or the same LRR domain as the full-length protein and where the increase according to the invention of the pathogen resistance can be achieved in transgenic plants using these fragments.

The term "essentially identical enzymatic activity" of the protein with kinase activity used in the method according to the invention means that the enzymatic activity in comparison with the enzymes encoded by the sequence with SEQ ID No. 1 or its derivatives is still at least 50%, preferably at least 60%, especially preferably at least 70%, particularly preferably at least 80% and most preferably at least 90%. Thus, proteins with kinase activity with an essentially identical enzymatic activity are also suitable for bringing about an increased pathogen resistance in transgenic plants.

The kinase activity of proteins can be determined by simple methods which are known to the skilled worker and which are referred to as kinase assays. To this end, for example, the protein which is to be tested, and which has been purified by, for example, immunoprecipitation, is incubated in a suitable buffer with radiolabeled ATP as the phosphate donor and with a suitable substrate, and the substrate is subsequently separated via an SDS-polyacrylamide gel. If the protein has a kinase activity, the substrate is labeled with the radiolabeled phosphate, and the radioactivity can be detected and quantitatively determined using suitable methods.

The increase according to the invention of the pathogen resistance can also be achieved by manipulating the expression of the plant-intrinsic endogenous protein, which corresponds to the protein according to the invention. This is, thus, a plant-intrinsic protein which confers an increase of the pathogen resistance which preferably has a kinase activity or an LRR domain, and especially preferably both traits. This manipulation of the protein expression can be achieved for example by modifying the promoter DNA sequence of the protein-encoding gene. Such a modification, which results in a modified, preferably increased, expression rate of the endogenous gene according to the invention, can be effected by deleting or inserting DNA sequences. A modification of the promoter sequence of endogenous genes according to the invention will, as a rule, lead to a modification of the expressed amount of the gene and thus, for example, also to a modification of the kinase activity which can be detected in the cell, or in the plants (if the protein has kinase activity). The modification of the promoter sequence of the endogenous gene according to the invention can also lead to a modification of the amount of protein with an LRR domain in the cell.

Another possibility for increasing the activity and the content of the endogenous protein according to the invention is to regulate transcription factors which are involved in the transcription of the respective endogenous gene according to the invention, for example by overexpression. The measures for overexpressing transcription factors are known to the skilled worker and are also disclosed for proteins according to the invention within the scope of the present invention.

Furthermore, an increased expression of an endogenous gene according to the invention can be achieved by a regulator protein which does not occur in the untransformed organism interacting with the promoter of these genes. Such a regulator can take the form of a chimeric protein which consists of a DNA binding domain and a transcription activator domain, as described, for example, in WO 96/06166.

Regarding a nucleic acid sequence, an expression cassette or a vector comprising said nucleic acid sequence or an organism transformed with said nucleic acid sequence, expression cassette or vector, "transgenic" means, for example, all those constructs or organisms which exist as a result of recombinant methods and in which either

a) the RNR8 nucleic acid sequence, or

b) a genetic control sequence, for example a promoter, which is operably linked with the RNR8 nucleic acid sequence, or

c) (a) and (b)

are not in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to be, for example, a substitution, addition, deletion, or insertion of one or more nucleotide residues. "Natural genetic environment" means the natural chromosomal locus in the organism of origin or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp. A naturally occurring expression cassette - for example the naturally occurring combination of the RNR8 promoter with the corresponding RNR8 gene – becomes a transgenic expression cassette when it is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenization. Such methods are described (US 5,565,350; WO 00/15815).

Within the scope of the present invention, "introduction" means all methods which are suitable for introducing an RNR8 nucleic acid sequence directly or indirectly into a plant or a

cell, compartment, tissue, organ or seed thereof, or for generating it therein. The introduction can lead to a transient or to a stable presence of an RNR8 nucleic acid sequence.

For example, "introduction" comprises methods such as transfection, transduction or transformation.

The introduction, into an organism or cells, tissues, organs, parts or seeds of the same (preferably into plants or plant cells, tissues, organs, parts or seeds), of an expression cassette according to the invention can advantageously be carried out using vectors in which the expression cassettes are present. The expression cassette can be introduced into the vector (for example a plasmid) via a suitable restriction cleavage site. The resulting plasmid is first introduced into *E. coli* cells. Correctly transformed *E. coli* cells are selected, cultured, and the recombinant plasmid is obtained by methods known to the skilled worker. The cloning step may be verified by restriction analysis and sequencing.

The vectors may take the form of, for example, plasmids, cosmids, phages, viruses or else agrobacteria. In an advantageous embodiment, the expression cassette is introduced by means of plasmid vectors. Preferred vectors are those which make possible a stable integration of the expression cassette into the host gene.

The generation of a transformed organism (or of a transformed cell) requires that the relevant DNA molecule is introduced into the relevant host cell and that the corresponding RNAs and proteins are subsequently formed by gene expression.

A multiplicity of methods (Keown et al. (1990) Methods in Enzymology 185: 527-537) are available for this procedure, which is referred to as transformation (or transduction or transfection). Thus, for example, the DNA or RNA can be introduced directly by microinjection or else by bombardment with DNA-coated microparticles. Also, the cell can be

permeabilized chemically, for example using polyethylene glycol, with the result that said DNA may enter the cell ends by diffusion. The DNA may also be introduced into the cell by means of protoplast fusion with other DNA-comprising units such as minicells, cells, lysosomes or liposomes. A further suitable method of introducing DNA is electroporation, where the cells are permeabilized reversibly by an electrical pulse. Suitable methods have been described (for example in Bilang et al. (1991) Gene 100: 247-250; Scheid et al. (1991) Mol. Gen. Genet. 228: 104-112; Guerche et al. (1987) Plant Science 52: 111-116; Neuhause et al. (1987) Theor. Appl. Genet. 75: 30-36; Klein et al. (1987) Nature 327: 70-73; Howell et al. (1980) Science 208: 1265; Horsch et al.(1985) Science 227: 1229-1231; DeBlock et al. (1989) Plant Physiology 91: 694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

In plants, the methods described are exploited for the transformation and regeneration of plants from plant tissue or plant cells for the purpose of transient or stable transformation. Suitable methods are mainly protoplast transformation by means of polyethylene-glycol-induced DNA uptake, the biolistic method with the gene gun, the method known as the particle bombardment method, electroporation, the incubation of dry embryos in DNA-comprising solution, and microinjection.

Besides these "direct" transformation techniques, a transformation may also be carried out by bacterial infection by means of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. The methods are described for example in Horsch et al. (1985) Science 225: 1229f.

If Agrobacteria are used, the expression cassette must be integrated into specific plasmids, which may either take the form of a shuttle or intermediate vector or of a binary vector. If a Ti or Ri plasmid is used for the transformation, at least the right border, but in most cases both the right and the left borders, of the Ti or Ri plasmid T-DNA is linked in the form of a

flanking region with the expression cassette to be introduced.

It is preferred to use binary vectors. Binary vectors are capable of replication both in *E. coli* and in Agrobacterium. As a rule, they comprise a selection marker gene and a linker or polylinker flanked by the right and left T-DNA border sequences. They can be transformed directly into Agrobacterium (Holsters et al. (1978) Mol. Gen. Genet. 163: 181-187). The selection marker gene, for example the nptII gene, which confers resistance to Kanamycin, permits a selection of transformed Agrobacteria. The Agrobacterium which acts as the host organism in this case should already comprise a Helfer Ti-plasmid with the vir region, which is required for the transfer of the T-DNA into the plant cell. An Agrobacterium thus transformed can be used for transforming plant cells. The use of T-DNA for the transformation of plant cells has been researched intensively and is described in (EP 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasserdam, Chapter V; An et al. (1985) EMBO J 4: 277-287). A variety of binary vectors are known, some of which are commercially available, such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA).

In the case of injection or electroporation of DNA or RNA into plant cells, the plasmid used does not need to meet any particular requirements. It is possible to use simple plasmids, such as those from the pUC series. If intact plants are to be regenerated from the transformed cells, it is required that an additional selectable marker gene is located in the plasmid.

Stably transformed cells, i.e. those which comprise the introduced DNA integrated into the DNA of the host cell, can be distinguished from untransformed cells when a selectable marker is part of the introduced DNA (McCormick et al. (1986) Plant Cell Reports 5: 81-84). For example, any gene which is capable of conferring resistance to antibiotics or herbicides (such as kanamycin, G 418, bleomycin, hygromycin or phosphinothricin), may be used as a marker. Transformed cells which express such a marker gene are capable of surviving the presence of

concentrations of a relevant antibiotic or herbicide which kill an untransformed wild type. Examples comprise the bar gene, which confers resistance to the herbicide phosphinothricin (Rathore et al. (1993) Plant Mol Biol 21(5): 871-884), the nptII gene, which confers resistance to kanamycin, the hpt gene, which confers resistance to hygromycin, or the EPSP gene, which confers resistance to the herbicide glyphosate. The resulting plants can be bred and hybridized in the customary manner. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

The abovementioned methods are described, for example, in Jenes et al. (1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by SD Kung and R Wu, Academic Press, p. 128-143 and in Potrykus (1991) Annu. Rev. Plant Physiol. Plant Molec. Biol. 42: 205-225). It is preferred to clone the construct to be expressed into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example into pBin19 (Bevan et al. (1984) Nucl. Acids Res. 12: 8711f).

As soon as a transformed plant cell has been generated, an intact plant can be obtained using methods known to the skilled worker. The starting material here is, for example, callus cultures. It is possible to induce, from these as yet undifferentiated cell biomasses, the formation of shoot and root in the known manner. The plantlets obtained can be planted out and used for cultivation.

The person skilled in the art also knows methods for regenerating plant parts and intact plants from plant cells. Methods for this purpose are described, for example, by Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al (1995) Plant Cell Rep. 14: 273-278; Jahne et al. (1994) Theor. Appl. Genet. 89: 525-533.

A further subject of the present invention is a recombinant nucleic acid molecule comprising the following elements in 5'-3' orientation:

- regulatory sequences of a promoter which is active in plant cells,
- a DNA sequence according to the invention in operable linkage thereto,
- if appropriate, regulatory sequences which may act as transcription, termination and/or polyadenylation signals in the plant cell, in operable linkage thereto.

"In operable linkage" means that a promoter and the nucleic acid sequence to be expressed and, if appropriate, further regulatory elements are arranged in such a way that each of the regulatory elements can fulfill its function when the nucleic acid sequence is expressed. To this end, direct linkage in the chemical sense is not necessarily required. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence from positions which are further away, or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as the promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 200 base pairs, especially preferably less than 100 base pairs and most preferably less than 50 base pairs.

Operable linkage, and a recombinant nucleic acid molecule, can be generated by means of customary recombination and cloning techniques as are described, for example, in Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML and Enquist LW (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Ausubel FM et al. (1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience and in Gelvin et al. (1990) In: Plant Molecular Biology Manual. However, further sequences which, for example, act as a linker with specific cleavage sites for restriction enzymes, or as a signal peptide, may also be positioned between

the promoter nucleic acid molecule to be expressed. The insertion of sequences may also lead to the expression of fusion proteins. Preferably, the recombinant nucleic acid molecule, comprising an operable linkage of at least a promoter and the nucleic acid sequence to be expressed, can exist in a vector-integrated form and be inserted into a plant genome, for example by transformation.

The term plant-specific promoters is understood as meaning, in principle, any promoter which is capable of governing the expression of genes, in particular foreign genes, in plants or plant parts, plant cells, plant tissues, or plant cultures. Here, expression may be for example, constitutive, inducible or development-dependent.

The following are preferred:

a) Constitutive promoters

"Constitutive" promoter is understood as meaning those promoters which ensure expression in a large number of, preferably all, tissues over a substantial period of plant development, preferably at all stages of plant development. In particular a plant promoter or a promoter derived from a plant virus are preferably used. Particularly preferred is the promoter of the CaMV cauliflower mosaic virus 35S transcript (Franck et al. (1980) Cell 21: 285-294; Odell et al. (1985) Nature 313: 810-812; Shewmaker et al. (1985) Virology 140: 281-288; Gardner et al. (1986) Plant Mol Biol 6: 221-228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J. 8: 2195-2202). Another suitable constitutive promoter is the "Rubisco small subunit (SSU)" promoter (US 4,962,028), the Agrobacterium nopaline synthase promoter, the TR dual promoter, the Agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29: 637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18: 675-689; Bruce et al. (1989) Proc. Natl. Acad. Sci. USA 86: 9692-9696), the Smas promoter, the cinnamyl

alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker. Especially preferred as constitutive promoter is the promoter of the nitrilase-1 (nit1) gene from *A. thaliana* (GenBank Acc. No.: Y07648.2, Nukleotide 2456-4340, Hillebrand et al. (1996) Gene 170:197-200).

b) Tissue-specific promoters

In one embodiment, promoters with specificity for the anthers, ovaries, flowers, leaves, stems, roots and seeds are used.

Seed-specific promoters are such as, for example, the phaseolin promoter (US 5,504,200; Bustos et al. (1989) Plant Cell 1(9): 839-53), the 2S albumin gene promoter (Joseffson et al. (1987) J. Biol. Chem. 262: 12196-12201), the legumin promoter (Shirsat et al. (1989) Mol. Gen. Genet. 215(2): 326-331), the USP (unknown seed protein) promoter; Bäumlein et al. (1991) Mol. Gen. Genet. 225(3): 459-67), the napin gene promoter (US 5,608,152; Stalberg et al. (1996) L. Planta 199: 515-519), the promoter of the gene coding for sucrose binding protein (WO 00/26388) or the legumin B4 promoter (LeB4; Bäumlein et al. (1991) Mol. Gen. Genet. 225: 121-128; Bäumlein et al. (1992) Plant Journal 2(2): 233-9; Fiedler et al. (1995) Biotechnology (NY) 13(10): 1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980). Further suitable seed-specific promoters are those of the genes coding for the high-molecular-weight glutenin (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase). Further preferred promoters are those which permit seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. The following can be employed advantageously: the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promoters described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the zein

gene, the kasirin gene, or the secalin gene).

Tuber-, storage-root- or root-specific promoters are, for example, the patatin promoter class I (B33), the potato cathepsin D inhibitor promoter.

Leaf-specific promoters are, for example, the potato cytosolic FBPase promoter (WO 97/05900), the SSU promoter (small subunit) of Rubisco (ribulose-1,5-bisphosphate carboxylase) or the ST-LSI promoter from potato (Stockhaus et al. (1989) EMBO J 8: 2445-2451). Epidermis-specific promoters are, for example, the OXLP gene (oxalate-oxidase-like protein) promoter (Wei et al. (1998) Plant Mol Biol 36: 101-112), a promoter consisting of the GSTA1 promoter and WIR1a intron (WO 2005/035766) and the GLP4 promoter (PCT/EP 2006/062747).

Other tissue-specific promoters are, for example, flower-specific promoters such as, for example, the phytoen synthase promoter (WO 92/16635) or the promoter of the Prr gene (WO 98/22593), and anther-specific promoters such as the 5126 promoter (US 5,689,049, US 5,689,051), the global promoter and the γ-zein promoter.

c) Chemically inducible promoters

The expression cassettes can also comprise a chemically inducible promoter (review article: Gatz et al. (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 89-108), by which the expression of the exogenous gene in the plant at a particular point in time can be controlled. Such promoters such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol. Biol. 22: 361-366), a salicylic-acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J 2: 397-404), an abscisic-acid-inducible promoter (EP 0 335 528) or an ethanol- or cyclohexanone-inducible promoter (WO 93/21334) can likewise be used.

d) Stress or pathogen-inducible promoters

Very especially advantageous is the use of pathogen-inducible promoters since these make possible expression only when required (i.e. infection with pathogens).

Thus, promoters which are used in one embodiment in the method according to the invention are active promoters, which are pathogen-inducible promoters.

Pathogen-inducible promoters comprise the promoters of genes which are induced as a consequence of infection by pathogens, such as, for example, genes of PR proteins, SAR proteins, β-1,3-glucanase, chitinase and the like (for example Redolfi et al. (1983) Neth. J. Plant Pathol. 89: 245-254; Uknes et al. (1992) Plant Cell 4: 645-656; Van Loon (1985) Plant Mol. Virol. 4: 111-116; Marineau et al. (1987) Plant Mol. Biol. 9: 335-342; Matton et al. (1987) Molecular Plant-Microbe Interactions 2: 325-342; Somssich et al. (1986) Proc. Natl. Acad. Sci USA 83: 2427-2430; Somssich et al. (1988) Mol. Gen. Genetics 2: 93-98; Chen et al. (1996) Plant J 10: 955-966; Zhang and Sing (1994) Proc. Natl. Acad. Sci. USA 91: 2507-2511; Warner et al. (1993) Plant J. 3: 191-201; Siebertz et al. (1989) Plant Cell 1: 961-968).

Also comprised are wound-inducible promoters such as that of the pinII gene (Ryan (1990) Ann. Rev. Phytopath 28: 425-449; Duan et al. (1996) Nat. Biotech. 14: 494-498), of the wun1 and wun2 gene (US 5,428,148), of the win1 and win2 gene (Stanford et al. (1989) Mol. Gen. Genet. 215: 200-208), of the systemin gene (McGurl et al. (1992) Science 225: 1570-1573), of the WIP1 gene (Rohmeier et al. (1993) Plant Mol. Biol. 22: 783-792; Eckelkamp et al. (1993) FEBS Letters 323: 73-76), of the MPI gene (Corderok et al. (1994) Plant J 6(2): 141-150) and the like.

A source for further pathogen-inducible promoters is the PR gene family. A series of elements

in these promoters have proven to be advantageous. Thus, the nucleotide region from nucleotide –364 to nucleotide –288 in the PR-2d promoter confers salicylate specificity (Buchel et al. (1996) Plant Mol. Biol. 30: 493-504). The sequence 5'-TCATCTTCTT-3' occurs repeatedly in the promoter of the barley β-1,3-glucanase and in more than 30 further stress-induced genes. In tobacco, this region binds a nuclear protein whose quantity is increased by salicylate. The PR-1 promoters from tobacco and Arabidopsis (EP-A 0 332 104, WO 98/03536) are also suitable as pathogen-inducible promoters. Preferred, since they are induced particularly specifically by pathogens, are the "acidic *PR-5"* (aPR5) promoters from barley (Schweizer et al. (1997) Plant Physiol. 114: 79-88) and wheat (Rebmann et al. (1991) Plant Mol. Biol. 16: 329-331). aPR5 proteins accumulate in approximately 4 to 6 hours after infection with pathogens and only show very little background expression (WO 99/66057). An approach for achieving an increased pathogen-induced specificity is the generation of synthetic promoters from combinations of known pathogen-responsive elements (Rushton et al. (2002) Plant Cell 14: 749-762; WO 00/01830; WO 99/66057). Further pathogen-inducible promoters from different species are known to the skilled worker (EP-A 1 165 794; EP-A 1 062 356; EP-A 1 041 148; EP-A 1 032 684).

Further pathogen-inducible promoters comprise the flacks *Fis1* promoter (WO 96/34949), the Vst1 promoter (Schubert et al. (1997) Plant Mol. Biol. 34: 417-426) and the EAS4 sesquiterpene cyclase promoter from tobacco (US 6,100,451).

Further preferred promoters are those which are induced by biotic or abiotic stress, such as, for example, the pathogen-inducible promoter of the PRP1 gene (or *gst1* promoter), for example from potato (WO 96/28561; Ward et al. (1993) Plant Mol. Biol. 22: 361-366), the heat-inducible hsp70 or hsp80 promoter from tomato (US 5,187,267), the chill-inducible alpha-amylase promoter from potato (WO 96/12814), the light-inducible PPDK promoter or the wound-induced pinII promoter (EP-A 0 375 091).

e) Mesophyll-tissue-specific promoters

"Mesophyll tissue" means the leaf tissue between the layers of the epidermis, consisting of the palisade tissue, the spongy tissue and the leaf veins.

One embodiment of the method according to the invention employs mesophyll-tissue-specific promoters such as, for example, the promoter of the wheat germin 9f-3.8 gene (GenBank Acc.-No.: M63224) or the barley GerA promoter (WO 02/057412). Said promoters are especially advantageous since they are both mesophyll-tissue-specific and pathogen-inducible. Further suitable is the mesophyll-tissue-specific Arabidopsis CAB-2 promoter (GenBank Acc.-No.: X15222) and the *Zea mays* PPCZm1 promoter (GenBank Acc.-No.: X63869) or homologues thereof. Mesophyll-tissue-specific means that the transcription of a gene is limited as a result of the specific interaction of Cis elements present in the promoter sequence and transcription factors binding to these elements and is limited to the smallest possible amount of plant tissue comprising mesophyll tissue; preferably, it means transcription limited to the mesophyll tissue.

Further mesophyll-specific promoters are PPCZm1 (=PEPC; Kausch (2001) Plant Mol. Biol. 45: 1-15); OsrbcS (Kyozuka et al. (1993) Plant Phys. 102: 991-1000); OsPPDK, acc. AC099041; TaGF-2.8, acc. M63223 (Schweizer (1999) Plant J. 20: 541-552); TaFBPase, acc. X53957;.TaWIS1, acc. AF467542 (US 2002/115849); HvBIS1, acc. AF467539 (US 2002/115849); ZmMIS1, acc. AF467514 (US 2002/115849); HvPR1a, acc. X74939 (Bryngelsson et al. (1994) Molecular Plant-Microbe Interactions 7(2): 267-75; HvPR1b, acc. X74940 (Bryngelsson et al. (1994) Molecular Plant-Microbe Interactions 7(2): 267-75); HvB1,3gluc; acc. AF479647; HvPrx8, acc. AJ276227 (Kristensen et al (2001) Molecular Plant Pathology 2(6): 311-317; and HvPAL, acc. X97313 (Wei (1998) Plant Molecular Biology 36: 101-112).

f) Epidermis-specific promoters

"Epidermis tissue" or epidermis means the outermost tissue layers of plants. The epidermis can have one or more layers; epidermis-"enriched" gene expression exists, such as, for example, that of Cer3, which may act as marker (Hannoufa. (1996) Plant J. 10 (3): 459-467).

By "epidermis", the skilled worker preferably means the prevailing epidermal tissue of primary aerial plant parts, for example of the shoot, of the leaves, flowers, fruits and seeds.

Examples of epidermis-specific promoters are WIR5 (=GstA1), acc. X56012 (Dudler & Schweizer, unpublished); GLP4, acc. AJ310534 (Wei (1998) Plant Molecular Biology 36: 101-112); GLP2a, acc. AJ237942 (Schweizer (1999). Plant J 20: 541-552); Prx7, acc. AJ003141 (Kristensen (2001) Molecular Plant Pathology 2(6): 311-317); GerA, acc. AF250933 (Wu (2000) Plant Phys. Biochem. 38: 685-698); OsROC1, acc. AP004656; RTBV, acc. AAV62708, AAV62707 (Klöti (1999) PMB 40: 249-266) and Cer3 (Hannoufa (1996) Plant J. 10 (3): 459-467).

g) Development-dependent promoters

Further suitable promoters are, for example, fruit-maturation-specific promoters such as, for example, the tomato fruit-maturation-specific promoter (WO 94/21794, EP 409 625). Development-dependent promoters partly comprise the tissue-specific promoters, since individual tissues develop by nature in a development-dependent fashion.

Especially preferred are constitutive promoters, and also leaf- and/or stem-specific, pathogen-inducible, root-specific, mesophyll-tissue-specific promoters, with constitutive, pathogen-inducible, mesophyll-tissue-specific and root-specific promoters being most preferred.

Further promoters may further be operably linked with the nucleic acid sequence to be expressed, which promoters make possible expression in further plant tissues or in other organisms such as, for example, *E. coli* bacteria. Plant promoters which are suitable are, in principle, all the above-described promoters.

Further promoters which are suitable for expression in plants are described (Rogers et al. (1987) Meth. in Enzymol. 153: 253-277; Schardl et al. (1987) Gene 61: 1-11; Berger et al. (1989) Proc. Natl. Acad. Sci. USA 86: 8402-8406).

Moreover, the average person skilled in the art is capable of isolating further suitable promoters by means of routine methods. Thus, the person skilled in the art can identify for example further epidermis-specific regulatory nucleic acid elements, using customary methods of molecular biology, for example hybridization experiments or DNA-protein binding studies. Here, a first step consists in, for example, isolating the desired tissue from the desired organism, from which the regulatory sequences are to be isolated, and in isolating the total poly(A)$^+$ RNA therefrom and establishing a cDNA library. In a second step, those clones from the first library are identified, by means of hybridization, whose corresponding poly(A)$^+$ RNA molecules accumulate in the desired tissue only, which identification is carried out with the aid of cDNA clones which are based on poly(A)$^+$ RNA molecules from another tissue. Thereafter, promoters which have tissue-specific regulatory elements are isolated with the aid of these cDNAs which have been thus identified. Moreover, the person skilled in the art has available further PCR-based methods for the isolation of suitable tissue-specific promoters.

The nucleic acid sequences present in the expression cassettes or vectors according to the invention may be operably linked with further genetic control sequences, besides a promoter. The term "of the genetic control sequences" is to be understood broadly and means all those sequences which have an effect on the coming into existence, or the function, of the recombinant nucleic acid molecule according to the invention. For example, genetic control

sequences modify the transcription and translation in prokaryotic or eukaryotic organisms. Preferably, the expression cassettes according to the invention comprise a promoter with a specificity as described above 5'-upstream of the respective nucleic acid sequence to be expressed recombinantly, and, as additional genetic control sequence, a terminator sequence in 3'-downstream direction, and, if appropriate, further customary regulatory elements, each operably linked with the nucleic acid sequence to be expressed recombinantly.

Genetic control sequences also comprise further promoters, promoter elements or minimal promoters which are capable of modifying the expression-controlling properties. Thus, for example, the tissue-specific expression can, as a result of genetic control sequences, additionally take place as a function of certain stress factors. Such elements are described, for example, for water stress, abscisic acid (Lam E and Chua NH (1991) J. Biol. Chem. 266(26): 17131 -17135) and heat stress (Schoffl F et al. (1989) Mol. Gen. Genet. 217(2-3): 246-53).

In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the method according to the invention. Moreover, it is also possible advantageously to use synthetic promoters.

Genetic control sequences furthermore also comprise the 5'-untranslated regions, introns or the noncoding 3' region of genes such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (general: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). It has been demonstrated that these may play a significant role in the regulation of gene expression. Thus, it has been demonstrated that 5'-untranslated sequences are capable of enhancing the transient expression of heterologous genes. An example of translation enhancers which may be mentioned is the 5'-leader sequence from the tobacco mosaic virus (Gallie et al. (1987) Nucl. Acids Res. 15: 8693-8711) and the like. They can furthermore promote tissue specificity (Rouster J et al. (1998) Plant J. 15: 435-440).

The recombinant nucleic acid molecule can advantageously comprise one or more so-called enhancer sequences in operable linkage with the promoter, which sequences make possible an enhanced transgenic expression of the nucleic acid sequence. Additional advantageous sequences may also be inserted at the 3' end of the nucleic acid sequences to be expressed recombinantly, such as further regulatory elements or terminators. The nucleic acid sequences to be expressed recombinantly may be present in the gene construct as one or more copies.

Polyadenylation signals which are suitable as control sequences are plant polyadenylation signals, preferably those which correspond essentially to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular of gene 3 of the T-DNA (octopine synthase) of the Ti plasmid pTiACHS (Gielen et al. (1984) EMBO J. 3: 835 ff) or functional equivalents thereof. Examples of especially suitable terminator sequences are the OCS (octopine synthase) terminator and the NOS (nopaline synthase) terminator.

Control sequences are furthermore to be understood as meaning those which make possible a homologous recombination or insertion into the genome of the host organism, or which permit the removal from the genome. In the case of homologous recombination, it is possible, for example, to replace the natural promoter of a specific gene with a promoter with specificity for the embryonal epidermis and/or the flower.

A recombinant nucleic acid molecule and a vector derived therefrom may comprise further functional elements. The term functional element is to be understood in a broad sense and means all those elements which have an effect on the generation, multiplication or function of the nucleic acid molecules, vectors or transgenic organisms according to the invention. Examples which may be mentioned, but not by way of limitation, are:

a) Selection markers which confer a resistance to a metabolism inhibitor such as 2-desoxyglucose 6-phosphate (WO 98/45456), antibiotics or biocides, preferably

herbicides, for example kanamycin, G 418, bleomycin, hygromycin or phosphinothricin. Especially preferred selection markers are those which confer a resistance to herbicides. Examples which may be mentioned are: DNA sequences which code for phosphinothricin acetyltransferases (PAT), and inactivate glutamine synthase gene inhibitors (bar and pat gene), 5-enolpyruvylshikimate-3-phosphate synthase genes (EPSP synthase genes) which confer resistance to Glyphosat® (N-(phosphonomethyl)glycine), the gox gene, which codes for Glyphosat®-degrading enzymes (glyphosate oxidoreductase), the deh gene (coding for a dehalogenase which inactivates dalapon), sulfonylurea- and imidazolinone-inactivating acetolactate synthases and bxn genes which code for bromoxynil-degrading nitrilase enzymes, the aasa gene, which confers a resistance to the antibiotic spectinomycin, the streptomycin phosphotransferase (SPT) gene, which confers a resistance to streptomycin, the neomycin phosphotransferase (NPTII) gene, which confers a resistance to kanamycin or geneticidin, the hygromycin phosphotransferase (HPT) gene, which mediates a resistance to hygromycin, the acetolactate synthase gene (ALS), which mediates a resistance to sulfonylurea herbicides (for example mutated ALS variants with, for example, the S4 and/or Hra mutation).

b) Reporter genes which code for easily quantifiable proteins and ensure via an intrinsic color or enzymic activity an assessment of the transformation efficiency or of the location or timing of expression. Very particular preference is given in this connection to reporter proteins (Schenborn and Groskreutz (1999) Mol. Biotechnol. 13(1): 29-44) such as the green fluorescence protein (GFP) (Sheen et al. (1995) Plant Journal 8(5): 777-784; Haselhoff et al. (1997) Proc. Natl. Acad. Sci. USA 94(6): 2122-2127; Reichel et al. (1996) Proc. Natl. Acad. Sci. USA 93(12): 5888-5893; Tian et al. (1997) Plant Cell Rep. 16: 267-271; WO 97/41228; Chui et al. (1996) Curr Biol 6: 325-330; Leffel et al. (1997) Biotechniques. 23(5): 912-8), the chloramphenicoltransferase, a luciferase (Ow et al. (1986) Science 234: 856-859; Millar et al. (1992) Plant.Mol. Biol. Rep. 10: 324-414), the aequorin gene (Prasher et al. (1985) Biochem. Biophys. Res. Commun.

126(3): 1259-1268), the β–galactosidase, R-locus gene (codes for a protein which regulates the production of anthocyanin pigments (red coloration) in plant tissue and thus makes possible the direct analysis of the promoter activity without the addition of additional adjuvants or chromogenic substrates; Dellaporta et al., In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium, 11: 263-282, (1988), with ß-glucuronidase being very especially preferred (Jefferson et al., EMBO J. 1987, 6, 3901-3907).

c) Origins of replication which ensure multiplication of the expression cassettes or vectors according to the invention in, for example, *E. coli*. Examples which may be mentioned are ORI (origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook and Russell, see above).

d) Elements which are necessary for agrobacterium-mediated plant transformation, such as, for example, the right or left border of the T-DNA or the vir region.

To successfully select transformed cells, it is generally additionally required to introduce a selectable marker which confers a resistance to a biocide (for example a herbicide) to the successfully transformed cells, a metabolism inhibitor such as 2-deoxyglucose 6-phosphate (WO 98/45456) or an antibiotic, thereby permitting the selection of the transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5: 81-84).

The present invention furthermore relates to transgenic plant cells and transgenic plants which comprise a nucleic acid sequence according to the invention or a recombinant nucleic acid molecule according to the invention, and parts of the plants, transgenic crop products and transgenic propagation material of these plants, such as protoplasts, plant cells, calli, seeds, tubers, cuttings, and the transgenic progeny of this plant.

The plants are preferably those which belong to the family Poaceae, plants are especially preferably selected from among the plant genera Hordeum, Avena, Secale, Triticum, Sorghum, Zea, Saccharum and Oryza, very especially preferably plants are selected from among the genera *Hordeum vulgare* (barley), *Triticum aestivum* (wheat), *Triticum aestivum subsp.spelta* (spelt), Triticale, *Avena sativa* (oats), *Secale cereale* (rye), *Sorghum bicolor* (sorghum), *Zea mays* (maize), *Saccharum officinarum* (sugarcane) and *Oryza sativa* (rice).

However, the method according to the present invention is also suitable for dicotyledonous useful plants such as, for example, cotton, leguminoses such as pulses and in particular alfalfa, soybean, oil seed rape, tomato, sugar beet, potato, sunflower, ornamentals and trees. Further useful plants may be fruit (in particular apples, pears, cherries, grapes, citrus, pineapples and bananas), oil palms, tea bushes, cocoa bushes and coffee bushes, tobacco, sisal and, among medicinal plants, Rauwolfia and Digitalis. Especially preferred are the dicotyledonous plants sugar beet, oilseed rape, soybean, tomato, potato and tobacco. Further useful plants can be seen from the US patent No. 6,137,030.

The specific expression of the protein according to the invention in the plants according to the invention or in the plant cells according to the invention can be detected, and monitored, with the aid of traditional methods of molecular biology and biochemistry. The skilled worker is familiar with these techniques, and he is easily capable of selecting a suitable detection method, for example a Northern Blot analysis for detecting protein-specific RNA or for determining the accumulation level of protein-specific RNA, or a Southern Blot analysis or PCR analysis for detecting DNA sequences which code for a protein according to the invention. The probe or primer sequences used for this purpose can either be identical to the sequence shown in SEQ ID No. 1 or can feature a small number of deviations from this sequence.

Naturally, the method according to the invention can also be combined with other methods for increasing the pathogen resistance in transgenic plants. Thus, for example, it is possible to increase, by suitable methods, the polypeptide quantity, the activity or the function of one or more resistance factors selected from the group consisting of Bax inhibitor 1 protein from *Hordeum vulgare* (GenBank Acc.-No.: AJ290421), from *Nicotiana tabacum* (GenBank Acc.-No.: AF390556), rice (GenBank Acc.-No.: AB025926), Arabidopsis (GenBank Acc.-No.: AB025927) or tobacco and oilseed rape (GenBank Acc.-No.: AF390555, Bolduc et al. (2003) Planta 216: 377-386), ROR2 (for example from barley (GenBank Acc.-No.: AY246906)), SnAP34 (for example from barley (GenBank Acc.-No.: AY247208)) and/or lumenal binding protein BiP for example from rice (GenBank Acc.-No. AF006825). Equally, it is possible to reduce, by suitable methods, the polypeptide quantity, the activity or the function of one or more resistance factors selected from the group consisting of RacB (for example from barley (GenBank Acc.-No.: AJ344223)), CSL1 (for example from Arabidopsis (GenBank Acc.-No.: NM116593)), HvNaOX (for example from barley (GenBank Acc.-No.: AJ251717); EP 1 525 315), MLO (for example from barley (GenBank Acc.-No. Z83834); WO 98/04586, WO 00/01722, WO 99/47552), ARM1 (armadillo repeat protein; EP application number 05110468.5).

A further subject of the invention relates to the use of the transgenic organisms according to the invention and of the cells, cell cultures, parts – such as, for example, in the case of transgenic plant organisms, roots, leaves and the like -, and transgenic propagation material such as seeds or fruits for the preparation of foodstuffs or feeding stuffs, pharmaceuticals or fine chemicals.

In one embodiment, the invention furthermore relates to a process for the recombinant production of pharmaceuticals or fine chemicals in host organisms, where a host organism or a part thereof is transformed with one of the above-described recombinant nucleic acid molecules, and this nucleic acid molecule comprises one or more structural genes which code

for the desired fine chemical or which catalyze the biosynthesis of the desired fine chemical, the transformed host organism is cultured, and the desired fine chemical is isolated from the culture medium. This process can be applied broadly to fine chemicals such as enzymes, vitamins, amino acids, sugars, fatty acids, natural and synthetic flavorings, aroma substances and colorants. The production of tocopherols and tocotrienols and of carotenoids is especially preferred. Culturing the transformed host organisms and isolation from the host organisms, or from the culture medium, is carried out by processes known to the skilled worker. The production of pharmaceuticals such as, for example, antibodies or vaccines, is described in Hood and Jilka (1999). Curr. Opin. Biotechnol.10(4): 382-6; Ma and Vine (1999) Curr. Top. Microbiol. Immunol. 236: 275-92.

The identification of the leucine-rich-repeat-comprising proteins with a kinase domain, RNR8, from barley as gene which confers barley's resistance to *Blumeria graminis* isolates, and its use for conferring the pathogen resistance in transgenic plants or plant cells, will now be shown in what follows. The examples hereinbelow are not to be construed as limiting. The content of all of the references, patent applications, patents and published patent applications is incorporated here by way of reference.

Examples

Example 1: General cloning methods

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, linking of DNA fragments, transformation of *E. coli* cells, the culturing of bacteria and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (2001), see above.

Example 2: Sequence analysis of recombinant DNA

The sequencing of recombinant DNA molecules was carried out using a laser fluorescence DNA sequencer, from ABI, following the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74: 5463-5467).

Example 3: BAC screening for identifying the clone which comprises the sequence according to the invention

DNA pools from a barley BAC library (Yu et. al. (2000) TAG 101: 1093-99) were used for identifying the gene coding for the sequence according to the invention in barley. BAC clones which comprise the sequence according to the invention were identified by means of PCR using the primers 5' CTT TCG TGC TTA TGT GGG TGT GAC (SEQ ID NO: 4) and 5' CAT GAG GAG TCT GCA ATA AGG A (SEQ ID NO: 5).

The PCR method was chosen for its high sensitivity to detect the searched-for DNA sequence. The analysis was carried out in a reaction volume of 20 µl. The reaction mixture consisted of 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.1% Triton X-100, 0.2 mM dNTP; 2 mM $MgCl_2$, in each case 0.6 µM oligonucleotides and Taq polymerase (concentration in the reaction mixture: ~ 1U μl^{-1}). Either 10 ng of BAC pool DNA or 2 µl of bacterial culture (for colony PCR) were used per reaction mixture. Existing cDNA sequences served as the basis for deriving the oligonucleotides 5'GGA TTT GTC ACG TCC AAC CT (SEQ ID NO: 6) and 5'ATT GGC AAT TGT GAT AGC CC (SEQ ID NO: 7).

The BAC DNA to be amplified and the primers were initially introduced, and subsequently mixed with the PCR reaction mixture. To destroy and disrupt the bacteria in a colony PCR, the initially introduced mixture was heated for 5 min at 95°C before adding the PCR reaction mixture. An initial step of 5 min at 95°C was used for denaturing the double-stranded DNA.

The touch-down PCR reaction was carried out in the steps 30 s 95°C; 30 s 60 to 55°C and 60 s 72°C for the first 10 cycles. With each cycle, the temperature was reduced by 0.5°C (60 to 55°C). A further 30 cycles were carried out with the steps 30 s 95°C; 30 s 55°C and 60 s 72°C. To carry out the final chain elongation, the reaction was incubated for 5 min at 72°C before being cooled to, and kept constant at, a temperature of 20°C. Since it was expected that, at 189 bp, the reaction product was short, the PCR experiments were analyzed using 2.5% agarose gels in 0.5x TBE buffer.

Identified individual clones were subcloned in two steps for identifying gene and promoter. First, the BAC DNA of a single clone was isolated by means of a Qiagen column (Maxi-Kit; Qiagen; isolation in accordance with the manufacturer's protocol). 5 – 10 kbp fragments were generated from this BAC DNA by means of shearing (Hydroshear: Genomic Solutions), and the resulting ends were filled up with Klenow to give smooth ends (reaction as specified in the manufacturer's protocol). The selection of the fragment lengths was carried out using an 0.8% agarose gel in 0.5% TBE. The relevant fragment length range was excised from the gel, and the DNA was eluted from the agarose gel with the aid of the Qiagen Gel Extraction Kit (elution in accordance with the manufacturer's protocol). The eluted 5-10 kbp fragments were ligated into an EcoRV-linearized pBluescript II SK(-) vector with smooth dephosphorylated ends (restriction and dephosphorylation in accordance with the manufacturer's instructions) and transformed chemically/thermally into highly competent *E. coli* cells. Thereafter, the transformants were arranged randomly with the aid of a picking robot (Qpick, Genetix) and transferred into microtiter plates with LB medium.

Using PCR, the subfragment which comprises the gene of interest and which maximizes the length of the potential 5'-upstream region was selected by means of PCR. The selected subfragment was again sheared into 1-2 kbp fragments, ligated, transformed, and the clones were stored in microtiter plates (see above). Among the picked clones, 96 colonies were selected at random and sequenced using the TempliPhi protocol, in accordance with the

manufacturer's protocol. The sequences were assembled. The sequence information obtained was used for annotating the coding exons in comparison with known sequences of other organisms in order to determine the sequence according to the invention and its potential promoters.

Example 4: Subcloning of the RNR8 gene into pIPKTA9

In order to verify in greater detail whether the identified gene RNR8 is responsible for mediating pathogen resistance, this gene was subcloned into the vector pIPKTA9 using the following protocol:

1. Digestion of NBS-LRR BAC (No. 027N11) with *Psp* 1406 I and *Xma*JI. This excises almost the entire coding portion of the RNR8 gene.
2. Blunt-ending with Klenow.
3. Gel elution of the 11 kb band of step 2 using the Qiagen gel extraction kit.
4. Ligation of the 11 kb band NBS_LRR (step 3) into TA38 in the presence of *Swa* I (Douchkov et al. (2005) Molecular Plant-Microbe Interactions 18: 755-761.)
5. PCR of a 1 kb NBS-LRR fragment in order to complete the coding region of the gene at the 5' end (upstream). Thermal Ace Polymerase (produces blunt-ended fragments). Primer NBS-LRR F1: GCT GAA CCA ACC CGG GGA GAA ATA (SEQ ID NO: 8) Primer NBS-LRR F1: AGA TGA TCG GAA GAA CAG TGC AAC (SEQ ID NO: 9)
6. PCR purification (step 5) with MinElute plates.
7. Cloning of the 5' NBS-LRR PCR fragment (step 6) into pIPKTA9, cleaved by *Sma* I.
8. Digestion of the 11kb LRR in TA38 (step 4) with *Aar I* and *Not I.*
9. Gel elution of the 11 kb LRR of step 8.
10. Digestion of pIPKTA9_NBS_LRR_5' (step 7) with *Aar I* and *Not I.*
11. Gel elution ~ 3.5 kb vector band of step 10.
12. Ligation of the fragments of steps 7 and 11 with T4 DNA ligase (Fermentas).

13. Verification of the final construct pIPKTA9_NBS-LRR by sequencing.

Since the same effects were obtained with the construct pIPKTA9_NBS-LRR as with the BAC clone, it was concluded that RNR8 is the gene on BAC No. 027N11 which is responsible for the observed pathogen resistance (see, in this context, also figure 1).

Example 5: Transient expression in wheat by particle bombardment

The following construct mixture was introduced into wheat leaves using a gene gun (Bio-Rad, model PDS-1000/He, Hepta adapter) by means of biolistic transformation, following the method of Douchkov et al. (2005) Mol. Plant-Microbe Interact. 18: 755-761:

Plasmid	Reaction 1	Reaction 2	Reaction 3	Reaction 4
pUbiGUS (reporter gene construct)	7 µg/shot	7 µg/shot	7 µg/shot	7 µg/shot
pIPKTA9 (empty overexpression vector)	7 µg/shot	---	---	---
pIPKTA9_TaPERO	---		7 µg/shot	---
BAC 027N11	---	14 µg/shot		---
pIPKTA9_NBS LRR	---	---	---	7 µg/shot

For the DNA coating, 2.18 mg of gold particles (diameter 1.0 µm, particle density 25 mg ml^{-1} in 50% (v/v) glycerol) were mixed with 14-21 µg of "supercoiled" DNA for each shot, and treated with 1 M $Ca(NO_3)_2$ pH 10 in such a way that the final $Ca(NO_3)_2$ concentration was 0.5 M. After centrifuging and washing with 70% (v/v) ethanol, the particles were resuspended in 96% (v/v) ethanol and divided between the 7 macrocarriers. In a vacuum (3.6×10^3 Pa), the particles were introduced into in each case 7 leaf segments of 7-day-old wheat plants (variety Kanzler) by means of a helium pressure surge of 7.6×10^6 Pa. For the bombardment, the leaf

segments were placed into a Petri dish on 0.5% (w/v) Phytoagar which had been treated with 20 µg ml^{-1} benzimidazole. The leaves were subsequently incubated for 4 h at +20°C and in indirect daylight.

Example 6: Inoculation of the leaf segments

The bombarded leaves were transferred onto 1% (w/v) Phytoagar with 20 µg ml^{-1} benzimidazole in 20 x 20 cm polycarbonate dishes. The infection with wheat powdery mildew spores was carried out in an inoculation tower by shaking spores from severely infected wheat leaves into the tower. The inoculum density was around 200 spores/mm^2. After 5 min, the dishes were removed, sealed and incubated for 40-48 h at +20°C and in indirect daylight.

Example 7: Histochemical GUS detection

The leaves were infiltrated in vacuo with the GUS detection solution (10 mM EDTA, 1.4 mM $K_3[Fe(CN)_6]$, 1.4 mM $K_4[Fe(CN)_6]$, 0.1% (v/v) Triton X-100, 20% (v/v) methanol, 1 mg/ml 3-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, 100 mM sodium phosphate buffer, pH 7.0) and incubated overnight at +37°C. After the detection solution had been removed, the leaves were destained for 15 min at +20°C in a solution of 7.5% (w/v) trichloroacetic acid and 50% (v/v) methanol.

Light microscopy was carried out using a Zeiss Axiolab microscope at 200x magnification. The cell contents of cells with GUS expression are blue. Using quantitative microscopy, the number of GUS-stained cells and the number of GUS-stained cells which contain at least 1 haustorium of the wheat powdery mildew fungus were counted for each shot. The susceptibility index was calculated from the number of haustorium-containing GUS-positive cells/all GUS-positive cells.

The result of the inoculation experiment is shown in figure 1. It emerged that the expression of the protein according to the invention from the BAC clone or the expression vector pIPKTA9_NBS-LRR reduces the susceptibility of the wheat plants to wheat powdery mildew by approximately 50% in comparison with plants which have been transformed with the empty vector.

Figures

Figure 1: Transient complementation experiment in wheat with barley BAC clone 027N11 (Morex BAC library) which comprises the leucine-rich-repeat protein kinase Rnr8. Wheat leaves were co-bombarded with pUbiGUS and either pIPKTA9 (empty overexpression vector), BAC clone 027N11, pIPKTA9_NBS LRR (see example 4) or pIPKTA9:TaPERO (overexpression construct for a peroxidase as a positive control). The data shown are means and standard deviations from 2 individual experiments with in each case 2 parallel bombardments.

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CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

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

i) nucleic acid sequences comprising nucleotide sequences which correspond to the coding sequences of SEQ ID No. 1,

ii) nucleic acid sequences comprising nucleotide sequences which code for a protein with the amino acid sequence shown in SEQ ID No. 2,

iii) nucleic acid sequences comprising nucleotide sequences which have at least 60% sequence identity with the coding sequences of SEQ ID No. 1, and/or

iv) nucleic acid sequences comprising nucleotide sequences which, under stringent conditions, hybridize with a complementary strand of a nucleotide sequence of i) to iii),

which codes for a protein which confers an increased pathogen resistance, preferably an increased resistance to fungal pathogens, in plants.

2. The nucleic acid molecule as claimed in claim 1, where the nucleic acid sequence originates from *Hordeum vulgare.*

3. A recombinant protein which confers, in plants, an increased pathogen resistance, preferably an increased resistance to fungal pathogens, and which is encoded by a nucleic acid sequence as claimed in claim 1 or 2.

4. A recombinant nucleic acid molecule, comprising the following elements in 5'-3' orientation:

- regulatory sequences of a promoter which is active in plant cells,

- a DNA sequence according to claim 1 in operable linkage thereto,
- if appropriate, regulatory sequences which may act as transcription, termination and/or polyadenylation signals in the plant cell, in operable linkage thereto.

5. The recombinant nucleic acid molecule as claimed in claim 4, wherein the DNA sequence is expressed under the control of a constitutive promoter, preferably the 35S CaMV or ubiquitine promoter.

6. The recombinant nucleic acid molecule as claimed in claim 4, wherein the DNA sequence is expressed under the control of a tissue-specific promoter.

7. The recombinant nucleic acid molecule as claimed in claim 6, wherein the tissue-specific promoter is an epidermis-, mesophyll- or leaf-specific promoter.

8. The recombinant nucleic acid molecule as claimed in claim 4, wherein the DNA sequence is expressed under the control of an inducible promoter.

9. The recombinant nucleic acid molecule as claimed in claim 8, wherein the inducible promoter is a pathogen- or wound-inducible promoter.

10. A method of increasing the pathogen resistance in transgenic plants, wherein a DNA sequence as claimed in claim 1 or 2 is introduced into, and expressed in, a plant or plant cell.

11. The method as claimed in claim 10, wherein the method comprises the following steps:

a) generating a recombinant nucleic acid molecule as claimed in any one of claims 4 to 9,

b) transferring the recombinant nucleic acid molecule from a) into plant cells and, if appropriate, integrating it into the plant genome, and

c) regenerating plants from the transformed plant cells.

12. The method as claimed in claim 10 or 11, where the pathogen is a fungal pathogen.

13. The method as claimed in any one of claims 10 to 12, where the pathogen is a fungus selected from the group consisting of mildew, rust, Fusarium and/or Septoria fungi.

14. A transgenic plant cell, comprising a nucleic acid sequence as claimed in claim 1 or 2, or a recombinant nucleic acid molecule as claimed in any one of claims 4 to 9 or obtained in a method as claimed in any one of claims 10 to 13.

15. The transgenic plant cell as claimed in claim 14, which has an increased amount of the protein as claimed in claim 3 in comparison to wild-type cells.

16. The transgenic plant cell as claimed in claim 14 or 15, which has increased pathogen resistance which is increased in comparison to wild-type cells.

17. The transgenic plant cell as claimed in any one of claims 14 to 16, has increased fungal resistance in comparison to wild-type cells.

18. The transgenic plant cell as claimed in claim 17, which features an increased resistance to mildew, rust and/or Septoria fungi.

19. The transgenic plant cell as claimed in claim 18, which has an increased resistance to Formae speciales of mildew.

20. A transgenic plant, comprising a plant cell as claimed in any one of claims 14 to 19 or generated in a method as claimed in any one of claims 10 to 13, and parts of these plants, transgenic crop products and transgenic propagation material of these plants, such as protoplasts, plant cells, calli, seeds, tubers, cuttings, and the transgenic progeny of this plant.

21. The transgenic plant as claimed in claim 20,
wherein the transgenic plant is a monocotyledonous plant, preferably a plant which belongs to the genera Avena, Triticum, Secale, Hordeum, Oryza, Panicum, Pennisetum, Setaria, Sorghum, Zea and the like.

22. The transgenic plant as claimed in claim 20,
wherein the transgenic plant is a dicotyledonous plant, preferably cotton, leguminoses such as pulses and in particular alfalfa, soybean, oilseed rape, canola, tomato, sugar beet, potato, ornamentals, sunflower, tobacco and trees.

23. The use of a nucleic acid molecule as claimed in any one of claims 1, 2 or 4 to 9 for the generation of transgenic plants and plant cells with an increased pathogen resistance in comparison to wild-type plant cells.

24. The nucleic acid according to any one of claims 1, 2 or 4 to 9, a recombinant protein according to claim 3, a method according to any one of claims 10 to 13, a transgenic plants cell according to any one of claims 14 to 19, a transgenic plant according to any one of claims 20 to 22, the use according to claim 23, substantially as hereinbefore described.

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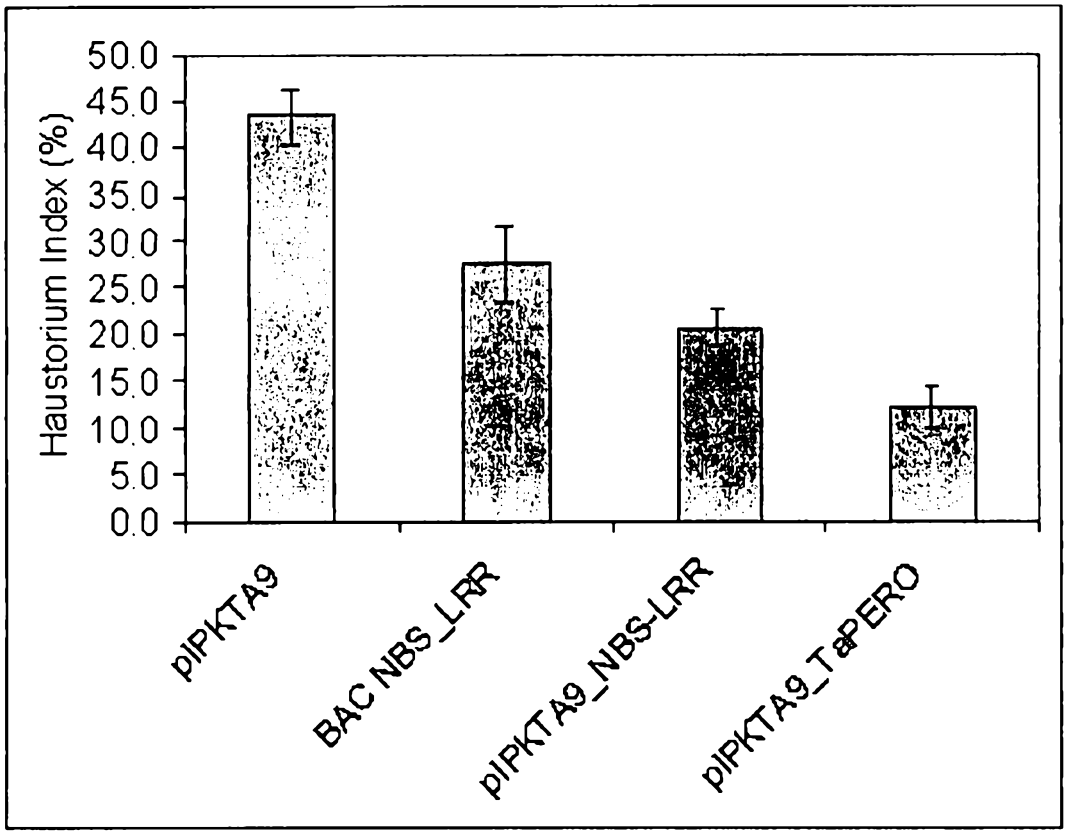
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pIPKTA9_TaPERO

Fig. 1

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