

The present invention relates to novel, recombinant deoxyribonucleic acids (DNA), to vectors and host organisms which contain them, and to transgenic plants which contain the recombinant DNA and exhibit increased
5 resistance toward pernicious organisms and plant diseases.

For a number of viruses, it has already been shown that when either structural or non-structural genes are isolated from these viruses and induced, in a suitable
10 manner, to express in transgenic plants, they give rise, in the plants, to increased resistance toward infection by the homologous virus or to attenuation and/or slowing down of symptom manifestation. This was demonstrated for the first time using the example of the coat protein of
15 tobacco mosaic virus (TMV) (EP-A2-0 223 452; Beachy et al., 1985; and Powell Abel et al., 1986). TMV belongs to the Tobamovirus genus and has a comparatively simple architecture and, for its genetic information, possesses, like many other plant viruses, a single-stranded RNA
20 molecule having plus-strand information (single-stranded positive-sense RNA genome). The genetic information is packaged in a coat which consists of many identical subunits. Later on, it was possible to show that a comparable strategy also succeeded in the case of the
25 tomato spotted wilt virus (TSWV), which is a virus which is essentially different and belongs to the Tospovirus genus of the Bunyaviridae family (EP-A1-0 426 195;

Gielen et al., 1991; De Haan et al., 1992). The genus
Tospovirus is classified as having a morphologically
spherical particle of about 100 nm in diameter and a
three-partite, single-stranded RNA genome, which, as a
5 nucleoprotein complex (nucleocapsid), is surrounded by a
lipid coat. This lipid coat is charged with
glycoproteins.

The three-partite genome possesses ambisense or minus-
strand information (single-stranded RNA molecules of
10 negative or ambisense polarity, De Haan et al., 1990). In
this case, resistance in transgenic plants is based on
transferring the N structural gene of a tospovirus, the
tomato spotted wilt virus (= TSWV) into a plant, where it
is expressed. The N gene product is a component of the
15 abovementioned nucleoprotein complex.

Whereas it was possible to demonstrate pronounced cross
resistance toward closely related tobamoviruses in the
case of the TMV coat protein (e.g. Tumer et al., 1987),
such cross resistance is not so far known in the case of
20 TSWV and expression of the N gene (De Haan et al., 1992;
MacKenzie and Ellis, 1992; Sheng-Zi et al., 1992).

Novel recombinant deoxyribonucleic acids (DNA) have been
found, which are characterized in that they consist of,
or contain, a combination of the following components or
25 components possessing a DNA which is in each case of
equal effect:

- (a) a double-stranded cDNA fragment which is derived from the RNA of the plum pox virus (PPV) ("fragment A"), and
- (b) a double-stranded cDNA fragment which is derived from the S RNA of tomato spotted wilt virus (TSWV) ("fragment B"), it being possible for this combination to also possess, in addition to further DNA fragments which may optionally be present, a promoter which is active in plant cells.

10 It has furthermore been found that transgenic plants which have the novel recombinant DNA integrated in their genome (and express this DNA) exhibit increased resistance toward pernicious organisms and plant diseases.

The transgenic plants also, and in particular, demonstrate increased resistance not only toward the homologous TSW virus isolate, from which fragment B was derived, but also toward other tospovirus species. This is surprising, since the recombinant deoxyribonucleic acids which are known from the state of the art and which were derived from naturally occurring viral genes do not have any such broad effect in the corresponding transgenic plants (De Haan et al., 1992; MacKenzie and Ellis, 1992). In addition to this, it is particularly surprising that increased resistance can be observed toward viruses which do not belong to the Tospovirus genus.

In the recombinant DNA according to the invention, fragment B is situated after (i.e. in the 3' direction)

fragment A, with the two fragments being connected to each other either directly or by an additional DNA fragment ("fragment C"). A promoter which is active in plants is placed upstream of fragment A when the DNA
5 according to the invention represents a complete transcription unit. In this case, fragment B is also followed by a 3' untranslated DNA which is connected to fragment B either directly or via an additional DNA fragment ("fragment D"). The 3' untranslated DNA contains a
10 transcription termination sequence (TTS) and a polyadenylation site.

The term "transcription termination sequence" denotes that this is a sequence which defines the end of the RNA synthesis, by a particular polymerase from a particular
15 DNA sequence serving as the template. In the case of the "chimeric genes" which are employed in transgenic plants, the TTS of the nopaline synthase gene (Zambryski et al., 1983), the octopine synthase gene (Herrera-Estrella et al., 1983) or the 35S transcript of CaMV (cauliflower
20 mosaic virus) (Pietrzak et al., 1986) may, for example, be used.

In addition, the term "polyadenylation site" or "polyadenylation signal" preferably denotes a sequence consisting of six nucleotides (e.g. AAUAAA) which effects
25 exact processing of the primary transcript and post-transcriptional synthesis of a "poly-A sequence" on the 3' end of the primary transcript.

From this, it follows, consequently, that a complete transcription unit has the following DNA arrangement:

(5') Promoter - fragment A - optionally: fragment C -
fragment B - optionally: fragment D - 3' untranslated DNA
5 (3')

The DNA arranged in this way is a part of the present invention. The following arrangements of recombinant DNA are likewise a constituent part of the present invention:

(5') Fragment A - optionally: fragment C - fragment B -
10 optionally: fragment D - 3' untranslated DNA (3'), and

Fragment A - optionally. fragment C - fragment B -
optionally: fragment D (3'), and

(5') Promoter - fragment A - optionally: fragment C -
fragment B (3')

15 Fragment A is a double-stranded cDNA which is derived from the RNA of a plum pox virus (PPV), the term "derived" denoting that the cDNA is a DNA which is present in a form complementary to the virus RNA.

A DNA as reproduced in SEQ ID No:7 is preferably used as
20 fragment A.

Fragment B is a double-stranded cDNA which is derived from S RNA, preferably from the N structural gene of

tomato spotted wilt virus (TSWV), preferably the L3 isolate, the term "derived" denoting that the cDNA is a DNA which is present in a form complementary to virus RNA. A DNA as reproduced in SEQ ID No:11 is preferably
5 used as fragment B.

Fragments A and B can be connected to each other either directly or, preferably, by a double-stranded DNA fragment C. A synthetic DNA fragment can be used as fragment C, which DNA fragment is selected such that it does not
10 contain a stop codon in any particular reading frame, and connects fragments A and B such that they form an uninterrupted open reading frame which begins in fragment A and ends at the end of fragment B and in this way encodes a polypeptide.

15 Fragment C can preferably contain up to 134 nucleotides. Particularly preferably, fragment C contains 2 to 68 nucleotides. The synthetic DNA whose sequence is reproduced in SEQ ID No:9 is very particularly preferred as fragment C. This fragment connects the open reading frame
20 beginning in fragment A with that of fragment B in a particularly advantageous manner.

All those promoters which are active in plants are suitable for use as promoters which can be located upstream of fragment A. Among these, those may be selected for special mention which are controlled by polymerase II, as may those which are under the control of
25 polymerase III. Promoters which originate from

phytopathogenic viruses are also selected for special mention. Among these, the 35S promoter of cauliflower mosaic virus (CaMV) is particularly preferred. Its sequence is reproduced in SEQ ID No:3. It is preferably
5 connected directly to fragment A.

All appropriate regions which bring about polyadenylation and transcription termination in plants can be employed as 3' untranslated DNA which contains a polyadenylation site and a TTS signal and follows fragment B, with
10 fragment D optionally being interpolated. 3' Untranslated DNA sequences from phytopathogenic viruses are preferably selected. The 3' untranslated CaMV 35S DNA, whose sequence is depicted in SEQ ID No:4, is particularly preferably used.

15 The above described fragments can in each case be replaced in the recombinant DNA according to the invention by corresponding fragments containing DNA having the same effect. In the description of this specification, and in the ensuing claims, DNA having the same effect is defined thus: 1 or more bases, or 1 or more codons, can be missing or be
20 replaced by other bases or codons, it being possible, however, to alter the fragments only to the extent that their effect is essentially preserved, i.e. that the combinations of fragments according to the invention lead in plants to elevated resistance toward pernicious organisms and plant diseases.

25 Any DNA can be considered as DNA having the same effect which can be hybridized under stringent conditions with a single-strand DNA according to SEQ ID No: 1-13 and which, in combination with the remaining



fragments, brings about the desired increase in resistance in plants. DNA having the same effect is preferably a DNA from which the same polypeptides, or polypeptides "having the same effect" can be "derived",
5 as from the DNA sequences according to SEQ ID: 1, 5, 7, 9 and 11 (cf. the protein sequences according to SEQ ID: 2, 6, 8, 10 and 12, as well). In this instance, derived denotes that a DNA can be transcribed into RNA, which can be translated into protein. Polypeptides having the same
10 effect means that they are polypeptides whose total primary sequence has the same effect, it being possible, however, to exchange individual amino acids without changing the secondary, tertiary or quaternary structure in such a way that the desired increase in resistance is
15 lost. DNA according to SEQ ID No:7 and 11 can also be employed as a probe in order to isolate appropriate additional fragments from plum pox viruses or tospoviruses that can be used in accordance with the invention. Using these probes, the fragments can also be
20 detected, in a customary manner, in vectors or in the plant genome.

In accordance with the invention, that recombinant DNA is preferred which contains at least one of the fragments A or B having the sequence according to SEQ ID No:7 or 11,
25 or a corresponding DNA having the same effect.

In accordance with the invention, that recombinant DNA is particularly preferred which contains both fragment A and fragment B having the sequences according to SEQ ID No:7 and 11, or a corresponding DNA having the same effect.

In accordance with the invention, that recombinant DNA is very particularly preferred which has the sequence according to SEQ ID No:1, including the corresponding DNA having the same effect. That recombinant DNA which has
5 the sequence according to SEQ ID No:5, or a corresponding DNA having the same effect, is very particularly preferred as a complete transcription unit.

The DNA fragments which are useable in accordance with the invention, as well as the DNA according to the
10 invention, can be obtained by the customary methods with the aid of the information in SEQ ID No:1-13. In addition to this, cDNA fragments of the plum pox virus (PPV) genome which are usable in accordance with the invention, as well as cDNA fragments of the S RNA genome of the TSW
15 virus, can be obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) (DSM) under the numbers PL-1080 (pPPV-NAT 5'-4) and PL-1081 (pTSW L3-308). Different cDNA's of the "Strassburg isolate" and of the
20 CM1841 isolate (ATCC No. 45031) of CaMV can likewise be obtained from the DSM under the numbers PL-1029 and PL-1033 (cf. DSM order list, plant virus group, Messeweg 11/12, W-3300 Braunschweig, Federal Republic of Germany).

The plasmids PL-1080 and PL-1081 have been deposited in
25 Escherichia coli at the German Collection of Microorganisms (DSM), Mascheroder Weg 1b, D-3300 Braunschweig, Federal Republic of Germany, in conformity with the regulations of the Budapest Treaty on the international

recognition of the deposition of microorganisms for the purposes of patent processes:

5	DSM reference designation	Entry Number	Date of deposition
	BY-1080 (contains PL-1080)	DSM 8276	07.05.1993
10	BY-1081 (contains PL-1081)	DSM 8277	07.05.1993

The recombinant DNA according to the invention does not occur in nature. It can be obtained by the person skilled in the art with the aid of the customary methods of molecular biology (Sambrook et al., 1989) using the information contained in the preceding description, in particular using SEQ ID No:1 to 13.

The present invention also relates to novel vectors, in particular plasmids or phages, which contain the recombinant DNA according to the invention, e.g. (as described below) the plasmids pSLTSWVL3 and pXLTSWVL3. The present invention also relates to novel host organisms, in particular microorganisms, which contain the recombinant DNA according to the invention, preferably integrated into the plasmid. The *Escherichia coli* and *Agrobacterium tumefaciens* strains which are customarily used are

particularly suitable for use as microorganisms. The *Agrobacterium tumefaciens* strain XLTSWVL3, which is described in more detail below and which contains the plasmid pXLTSWVL3, may be mentioned by way of example.

- 5 The vectors and microorganism strains can be obtained by the person skilled in the art with the aid of the customary methods of molecular biology and using the information contained in the preceding description, particularly using Seq ID No: 1 to 13.
- 10 The recombinant DNA according to the invention can be inserted into the genome of plants, as a result of which these transgenic plants are rendered more highly resistant toward pernicious organisms and plant diseases.

- The elevated resistance of the transformed plants is of importance for agriculture and forestry, for the cultivation of ornamental plants and medicinal plants, and for plant breeding. It is also advantageous in the cultivation of plant cells, e.g. for isolating pharmaceutically utilizable substances, to have plant cells available
- 15 which exhibit elevated resistance against infection by microbial pests, in particular fungi and viruses.
- 20

- Consequently, the present invention also relates to a process for preparing transgenic plant cells (including protoplasts) and plants (including plant parts and seeds)
- 25 having increased resistance to pernicious organisms and plant diseases, which process is characterized in that

- (a) the DNA according to the invention is introduced once or more than once into the genome of plant cells (including protoplasts) and, where appropriate,
- 5 (b) whole, transformed plants are regenerated from the transformed plant cells (including protoplasts) and, where appropriate, multiplied, and, where appropriate,
- 10 (c) the desired plant parts (including seeds) are isolated from the transgenic plants of the parental generation which result in this way, or from further generations obtained therefrom, and,
- 15 (d) where appropriate, the genome of the transgenic plants is, in order to produce further plants, combined with the genome of other plants, and the descendants multiplied.

The process steps (a), (b) and (c) can be carried out in a customary manner in accordance with the known processes and methods.

- 20 Transgenic plant cells (including protoplasts) and plants (including plant parts and seeds) which contain one or more copies of the DNA according to the invention, as well as such transformed plant cells and plants which can be obtained by the above processes, are likewise included
- 25 in the present invention.

- (a) The use of the recombinant DNA according to the invention for increasing the resistance of plants toward pernicious organisms and plant diseases,
- 5 (b) the use of the recombinant DNA according to the invention for producing plant cells, plants, plant parts and plant multiplication material having an elevated resistance toward pernicious organisms and plant diseases, and
- 10 (c) the use of the transgenic plants according to the invention (including plant parts and plant seeds) for producing multiplication material and for producing novel plants and their multiplication material

are also parts of the present invention.

- 15 Plants (including plant cells, plant parts and multiplication material) are also part of the present invention which contain the protein according to SEQ ID No: 2 or 6, respectively, or, where appropriate, contain a protein according to SEQ ID No: 2 or 6, respectively,
- 20 which is truncated at the aminotermminus.

A number of different methods are available for inserting the recombinant DNA according to the invention into the genetic material of plants or plant cells. The transfer of DNA can be effected in accordance with the customary

25 and well-known methods, the person skilled in the art

being able to ascertain without difficulty the appropriate method in each case. The DNA according to the invention, which is to be used for transforming plants, must contain an appropriate promoter which is active in plants
5 and a 3' untranslated region. The recombinant DNA according to SEQ ID No:5 is particularly suitable.

The Ti plasmid of *Agrobacterium tumefaciens* is available as a particularly favorable vector of wide applicability for transferring foreign DNA into the genomes of
10 dicotyledonous and monocotyledonous plants. The DNA according to the invention is, together with regulatory DNA sequences, inserted into the T DNA of appropriate Ti plasmids (e.g. Zambryski et al., 1983) and transferred by infection of the plant, infection of plant parts or plant
15 tissues, such as, for example, leaf disks, stalks, hypocotyls, cotyledons or meristems, or tissue derived therefrom, such as, for example, secondary embryos and calluses, or by coculturing protoplasts with *Agrobacterium tumefaciens*.

20 An alternative is to incubate the DNA in plant protoplasts (e.g. Hain et al., 1985; Krens et al., 1982; Paszkowski et al., 1984) in the presence of polycations or calcium salts and polyethylene glycol.

Uptake of the DNA can also be additionally encouraged by
25 using an electrical field (electroporation) (e.g. Fromm et al., 1986).

The DNA can also be introduced in a known manner by way of plant pollen, with the pollen being "bombarded" with physically accelerated particles which harbor the DNA (cf. EP-A 0 270 356).

- 5 The plants are regenerated in a known manner using appropriate nutrient media (e.g. Nagy and Maliga 1976).

In a preferred embodiment of the process according to the invention (according to the method from EP-A 116 718), the recombinant DNA according to the invention is cloned,
10 in isolated form, into a suitable intermediate E.coli vector, e.g. pGV700 or pGV710 (cf. EP-A 116 718), or preferably derivatives thereof which additionally contain a reporter gene such as nptII (Herrera-Estrella et al. 1983) or hpt (Van den Elzen et al. 1986), for example.

- 15 The plasmid constructed in this way is transferred by customary methods (e.g. Van Haute et al. 1983) into *Agrobacterium tumefaciens* which contains, for example, pGV 3850 or derivatives thereof (Zambryski et al. 1983). As an alternative, the DNA according to the invention can
20 be cloned in a binary vector, e.g. pCV001 or pCV002 (e.g. Koncz and Schell 1986), and then transferred, as described above, into a suitable *Agrobacterium* strain (Koncz and Schell 1986). The resulting *Agrobacterium* strain, which contains the recombinant DNA in a form
25 which is transferrable to plants, is subsequently used for the plant transformation.

In a further preferred embodiment, the recombinant DNA is transferred into plant protoplasts, where appropriate together with another plasmid which contains a reporter gene for plant cells, e.g. for kanamycin resistance (e.g. Herrera-Estrella et al. 1983) or a hygromycin resistance (van den Elzen, 1986), preferably pLGV neo 2103 (Hain et al., 1985), pMON 129 (Fraley R.T. et al., Proc. National Acad. Sci. USA 80, 4803 (1983)), pAK 1003, pAK 2004 (Velten J. et al., EMBO Journ. Vol. 3, 2723 (1984)) or pGSST neo 3 (pGSST3) (EP-A-189 707), by direct gene transfer in a customary manner (e.g. Hain et al 1985). In this context, the plasmid(s) can be present in circular form, although linear form is preferred. When a plasmid containing a reporter gene is used, kanamycin-resistant protoplasts are then examined for expression of the recombinant DNA. In the alternative case (without reporter gene) the resulting calluses are tested for expression of the recombinant DNA (screening using customary methods).

Transgenic plants or plant cells are produced in accordance with known methods, e.g. by transformation of leaf disks (e.g. Horsch et al. 1985), by coculture of regenerating plant protoplasts or cell cultures with *Agrobacterium tumefaciens* (e.g. Marton et al. 1979, Hain et al. 1985) or by direct DNA transfection. Transformed plants which result are detected either by selecting for expression of the reporter gene, e.g. by phosphorylating kanamycin sulfate in vitro (Reiss et al. 1984; Schreier et al. 1985) or by the expression of nopaline synthase

(in accordance with Aerts et al. 1983) or of the recombinant DNA according to the invention by Northern blot analysis and Western blot analysis. The gene products of the recombinant DNA can also be detected in transformed plants in a known manner using specific antibodies (Adam et al. 1987, 1991).

The transformed plant cells are cultivated and regenerated to form whole plants in accordance with the usual methods using the nutrient media which are appropriate in each case.

Both the transformed plant cells and the transformed plants, which contain the DNA according to the invention and which are constituent parts of the present invention, exhibit a considerably higher degree of resistance to pernicious organisms and plant diseases, in particular to nematodes, phytopathogenic fungi and viruses.

In connection with the present invention, the expression "plants" denotes both whole plants and plant parts, such as leaves, seeds, tubers, cuttings, etc. "Plant cells" include protoplasts, cell lines, plant calluses, etc. "Multiplication material" denotes plants, plant parts, such as seeds, tubers and cuttings, as well as plant cells, which can be used for multiplying the transgenic plants and plant cells, and is consequently likewise a part of the present invention.

Practically all plants are included in those plants which

can be imparted elevated resistance toward pests by the incorporation (transformation) of the recombinant DNA according to the invention. There is, naturally, a particular requirement for producing resistance in the

5 case of the cultivated plants, such as forestry plants, e.g. spruces, fir trees, Douglas firs, pines, larches, beech trees and oak trees, as well as plants which provide nutrients and raw materials, e.g. cereals (in particular wheat, rye, barley, oats, millet, rice and

10 maize), potatoes, leguminous plants (such as pulses and, in particular, alfalfa and soya beans), vegetables (in particular cabbage species and tomatoes), fruit (in particular apples, pears, cherries, grapes, citrus fruits, pineapples and bananas), oil palms, tea, cacao

15 and coffee bushes, tobacco, sisal and cotton, as well as in the case of medicinal plants, such as Rauwolfia and Digitalis. Potatoes, tomatoes, leguminous plants and Curcubitaceae may be particular preferably mentioned.

Animal pests, such as insects, mites and nematodes, as

20 well as microbial pests, such as phytopathogenic fungi, bacteria and viruses, may be mentioned as pests (pernicious organisms and organisms which elicit plant diseases) against which elevated resistances may be achieved according to the invention. Those which are

25 selected for special mention are plant-damaging nematodes and microbial pests, in particular phytopathogenic fungi and viruses.

The pernicious insects include, in particular insects of

the orders:

Orthoptera, Dermaptera, Isoptera, Thysanoptera, Heteroptera, Homoptera, Lepidoptera, Coleoptera, Hymenoptera and Diptera.

- 5 The pernicious mites include, in particular:
Tarsonemus spp., Panonychus spp. and Tetranychus spp.

- 10 The plant-parasitizing nematodes include Pratylenchus spp., Radopholus similis, Ditylenchus dipsaci, Tylenchulus semipenetrans, Heterodera spp., Globodera spp., Meloidogyne spp., Aphelenchoides spp., Longidorus spp., Xiphinema spp., Trichodorus spp.,

The microbial pests include, in particular, the phytopathogenic fungi:

- 15 Plasmodiophoromycetes, Oomycetes, Chytridiomycetes, Zygomycetes, Ascomycetes, Basidiomycetes, Deuteromycetes.

The phytopathogenic bacteria include, in particular the Pseudomonadaceae, Rhizobiaceae, Enterobacteriaceae, Corynebacteriaceae and Streptomyetaceae.

- 20 The virus diseases include, in particular, mosaic, dwarfing and yellowing viroses, and viroses which are elicited by tospoviruses.

Some causative agents of viral, fungal and bacterial

diseases which come under the generic names listed above may be mentioned as examples, but not by way of limitation:

- Barley yellow dwarf virus (BYDV), potato virus Y (PVY),
5 cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), tristeza virus, tobacco mosaic virus (TMV), tobacco necrosis virus (TNV), beet necrotic yellow vein virus (BNYVV) and, in particular, tomato spotted wilt virus (TSWV), all the tospoviruses, tobacco mosaic virus
10 (TMV) and other tobamoviruses, and tobacco rattle virus and other tobnaviruses.

Xanthomonas species, such as, for example, Xanthomonas campestris pv. oryzae;

- Pseudomonas species, such as, for example, Pseudomonas
15 syringae pv. lachrymans;

Erwinia species, such as, for example, Erwinia amylovora;

Pythium species, such as, for example, Pythium ultimum;

Phytophthora species, such as, for example, Phytophthora infestans;

- 20 Pseudoperonospora species, such as, for example, Pseudoperonospora humuli or Pseudoperonospora cubense;

Plasmopara species, such as, for example, Plasmopara viticola;

- Peronospora species, such as, for example, Peronospora
25 pisi or P. brassicae;

Erysiphe species, such as, for example, Erysiphe graminis;

- Sphaerotheca species, such as, for example, Sphaerotheca fuliginea;
- Podosphaera species, such as, for example, Podosphaera leucotricha;
- 5 Venturia species, such as, for example, Venturia inaequalis;
- Pyrenophora species, such as, for example, Pyrenophora teres or P. graminea (conidia form: Drechslera, syn: Helminthosporium);
- 10 Cochliobolus species, such as, for example, Cochliobolus sativus (conidia form: Drechslera, syn: Helminthosporium);
- Uromyces species, such as, for example, Uromyces appendiculatus;
- 15 Puccinia species, such as, for example, Puccinia recondita;
- Tilletia species, such as, for example, Tilletia caries;
- Ustilago species, such as, for example, Ustilago nuda or Ustilago avenae;
- 20 Pellicularia species, such as, for example, Pellicularia sasakii;
- Pyricularia species, such as, for example, Pyricularia oryzae;
- Fusarium species, such as, for example, Fusarium culmorum;
- 25 Botrytis species, such as, for example, Botrytis cinerea;
- Septoria species, such as, for example, Septoria nodorum;
- Leptosphaeria species, such as, for example, Leptosphaeria nodorum;

Cercospora species, such as, for example, Cercospora canescens;

Alternaria species, such as, for example, Alternaria brassicae;

- 5 Pseudocercospora species, such as, for example, Pseudocercospora herpotrichoides.

In addition, Helminthosporium carbonum may be listed.

Literature:

- 10 Adam G., Chagas C.M., Lesemann D.E.; (1987) *J. of Phyto-pathology* 120, 31-43

Adam G., Lesemann D.E., Vetten H.J. (1991) *Ann. appl. Biol.* 118, 87-114

Aerts M. et al., *Plant Sci. Lett.* 17 (1993), 43-50

- 15 Beachey R.N., Chen Z-L., Horsch R.B., Rogers S.G., Hoffmann N.J. and Fraley R.T. (1985) *EMBO J* 4, 3047-3050

de Avila A.C., de Haan P., Kormelink R., Resede RdeO., Goldbach R.W., and Peters D. (1993) *J. of Gen. Virol.* 74, 153-159

Chomezynski et al., *Anal. Bioch.* 162 (1987), 156-159

- 20 de Haan P., Wagemakers L., Peters D. and Goldbach R. (1990) *J. Gen. Virol.* 71, 1001-1007

de Haan P., Gielen J.J.L., Prins M., Wijkamp I.G., van Schepen A., Peters D., van Grinsven M.Q.J.M. and Goldbach R. (1992) *Bio/technologie* 10, 1133-1137

Fromm M.E. et al., *Nature* 319 (1986), 791-793

- 5 Gielen J.J.L., de Haan P., Kool A.J., Peters D., van Grinsven M.Q.J.M. and Goldbach R. (1991) *Bio/technologie* 9, 1363-1367

Goodall et al., *Meth. in Enzym.* 181 (1990), 148-161

Goodall and Filipowicz, *Cell* 58 (1989), 473-483

- 10 Herrea-Estrella L. et al., *Nature* 303 (1983), 209-213

Hain R. et al., *Molec. Gen. Genet.* (1985) 199, 161-168

Herrea-Estrella L. et al., *EMBO J2* (1983), 987-995

Hoekema A., Hirsch P.R., Hooykaas P.J.J. and Schilperoort R.A. (1983) *Nature* 303, 179-180

- 15 Horsch R.B. et al., *Science* 277 (1985), 1229-1231

Krens F.H. et al., *Nature* 296 (1982), 72-74

Koncz C. and Schell J., *Mol. Gen. Genet.* 204 (1986), 338-396

Landsmann J., Llewellyn D., Dennis E.S. and Peacock W.J.
(1988) *Mol. Gen. Genet.* 214, 68-73

MacKenzie D.J. and Ellis P.J. (1992) *Molecular Plant-Microbe Interactions* 5, 34-40

- 5 Maiss E., Timpe U., Brisske A., Jelkmann W., Casper R.,
Himmler G., Mattanovich D. and Katinger H.W.D. (1989) *J.
of Gen. Virol.* 70, 513-524

Maiss E., Ivanova L., Breyel E. and Adam G. (1991) *J. of
Gen. Virol.* 72, 461-464

- 10 Maiss E., Timpe U., Brisske-Rode A., Lesemann D-E. and
Casper R. (1992) *J. of Gen. Virol.* 73, 709-713

Marton L. et al., *Nature* (1979), 1229-1231

Mullis K.B. and Faloona F.A. (1987) *Methods Enzymol* 155,
335-350

- 15 Murashige T. and Skoog F., *Physiol. Plant.* 15 (1962), 47

Paszkowski J. et al., *EMBO J* 3 (1984), 2717-2722

Pietrzak et al. (1986) *Nucleic Acid Res.* 14, 5857-5868

Powell Abel P. et al. (1986) *Science* 232, 738-743

Reiss B. et al. , GENE 1081 (1984), 211-217

Sambrook J., Fritsch E.F. and Maniatis T. (1989) *Molecular Cloning a Laboratory Manual*, 2nd Edition

Schreier P. et al., EMBO J4 (1985), 25-32

5 Sheng-Zi et al. Phytopathology 82 (1992), 1223-1229

Steinecke et al., EMBO J11 (1992), 1525-1530

Töpfer R., Matzeit V., Gronenborn B., Schell J. and Steinbiss H.H. (1987) *Nucl. Acid Res.* 15, 5890

Tumer N.E., O'Connell K.M., Nelson R.S., Sanders P.R.,
10 Beachy R.N., Fraley R.T. and Shah D.M. (1987) *EMBO J*6,
1181-1188

Van den Elzen P.J.M. et al., *Plant Mol. Biol.* 5 (1985),
299-302

Van Haute E. et al., EMBO J2 (1983), 411-418

15 Velten J. et al., EMBO J12 (1984), 2723-2730

Zambryski et al., EMBO J. 2 (1983), 2143-2150.

The following examples are intended to clarify the present invention:

1. Construction of the recombinant DNA and transfer into agrobacteria:

As already explained, the present invention relates to novel recombinant DNA, in particular a recombinant chimeric gene (SEQ ID No: 1), which is under the control of a promoter and contains a 3'-terminal sequence. The CaMV35S promoter (5' region) (SEQ ID No: 3) and the 3' untranslated region (SEQ ID No: 4) of the CaMV35S transcript are used here by way of example (Töpfer et al. 1987 and EP-A2-0 223 452). The entire example used here is shown in SEQ ID No: 5. The DNA (chimeric gene) (SEQ ID No: 1) according to the invention consists of several pieces which are joined in a particular sequence. It consists of a double-stranded cDNA fragment of 152 base pairs which was derived from the RNA of plum pox virus (PPV) (Maiss et al., 1989). This corresponds to the viral RNA sequence at positions 1-152 (SEQ ID No: 7) (Maiss et al., 1989). In this fragment, an open reading frame begins which is connected via a synthetic cDNA fragment (SEQ ID No: 9) to a cDNA fragment of the S RNA of the L3 isolate of the TSWV N gene (Maiss et al.; 1991) (SEQ ID No: 11). This connection is such that an open reading frame is generated which has an overall length of 956 bases without interruptions and which ends at position 992 of "SEQ ID No: 1". The subsequent cDNA sequence of the S RNA of the 3' non-translated region of TSWV (SEQ ID No: 11) is

connected via a synthetic sequence (SEQ ID No: 13) to the 3' region of the 35S transcript of CaMV (SEQ ID No: 4) (Töpfer et al.; 1987).

5 The construction was effected using methods which are well-known and the details of which are described in (Sambrook, Fritsch and Maniatis, 1989).

10 The detailed procedure was as follows. A HindIII/-BamHI fragment of 454 base pairs in length from the commercially available plasmid pCaMVCN (Pharmacia) was cloned into the commercially available plasmid pT7T3 19U (Pharmacia) and termed pT7T3 35S. This novel plasmid was cut with SalI and the protruding ends filled in, and the DNA was then cut with EcoRI. A DraI/EcoRI fragment (572 base pairs in length), 15 from the plasmid pPPV-NAT 5'4 (Maiss et al., 1989; Maiss et al. 1992), was then cloned into the plasmid thus prepared. This fragment contains a cDNA copy of the 5' end of PPV. The new plasmid was termed p35S 5'.16.

20 Using the synthetic oligonucleotide 5'-TGTGTTGAGTTTTTATATTTTCCTCTCCAAATGAAA-3' (SEQ ID No: 14) which is complementary to the non-coding strand, the 3' end of the CaMV35S promoter sequence (5'-...AGAGG-3') (SEQ ID No: 15) was attached, with 25 the aid of *in-vitro* mutagenesis, to the extreme 5' end (5'-AAAATAT....-3') (SEQ ID No: 16) of the cDNA of the PPV sequence. The new plasmid which resulted

was termed p35S. A HincII fragment of 568 base pairs in length was isolated from this plasmid and cloned into the commercially available plasmid pT7T3 19U, which had been previously cut with SmaI and HincII. The designation of the resulting plasmid is pT7T3 19UPPVL. This plasmid was cut with EcoRV and SalI, and a fragment of 239 base pairs in length was isolated. This fragment was cloned into the EcoRV, XhoI cleavage sites of pRT101 (Töpfer et al. 1987). The resulting plasmid was termed pSL.

A BamHI/HindIII fragment was isolated from the plasmid pTSWV-L3/308 (Maiss et al.; 1991). The ends were filled in and inserted into the SmaI cleavage site of the vector pRT101 (Töpfer et al. 1987). The plasmid, which contains the inserted fragment in the correct orientation, was termed pSTSWVL3. An RsaI/-BamHI fragment of 868 base pairs in length was isolated from this plasmid, and cloned into the abovementioned vector pSL, which had been cut with SmaI and BamHI. The new plasmid which resulted was termed pSLTSWVL3. A fragment of 1716 base pairs in length, which contains the entire chimeric gene and the 5' and 3' untranslated regions of CaMV (35S), can be isolated from this latter plasmid using HindIII. This fragment was cloned into the plasmid pXL222 (Landsmann et al. 1988), which had been cut with HindIII. The resulting plasmid was termed pXLTSWVL3. This plasmid (pXLTSWVL3) was mobilized or transfected into the *A. tumefaciens* strain LBA 4404

(Hoekema et al. 1983). This new strain of Agrobacterium was termed XLTSWVL3.

2. Production of transgenic plants, and detection of the expression of the chimeric gene in these plants

5 The Agrobacterium strain XLTSWVL3 was used for transforming plant cells or plant parts.

10 The transgenic plants were selected on kanamycin-containing medium using a marker and selection gene, the gene for kanamycin resistance, which had been transferred at the same time. Twenty one individual candidates were chosen on the basis of their ability to express the chimeric gene. This involved firstly determining gene expression at the level of the mRNA. For this, the entire RNA isolated from transgenic plants was translated into cDNA using reverse transcriptase and the synthetic oligonucleotide 5'-GTC AGT GGC TCC AAT CCT GTC TGA AG-3' (SEQ ID No: 17), and the existence of mRNA of the chimeric gene was amplified with the aid of a second synthetic oligonucleotide 5' - TGT GGA GAA TTC GAG CTC GGT AC - 3' (SEQ ID No: 18) and the polymerase chain reaction (PCR; Mullis and Faloona, 1987), and identified as a fragment of 328 base pairs in length.

25 The twenty-one transgenic plants in which gene expression had been detected in the abovedescribed way and manner were next tested for production of

the chimeric gene product using a specific antibody (ELISA) (Adam et al., 1987; 1991). The candidates XL4, XL8, XL11, XL14, XL15 and XL19 gave a clearly measurable signal and were selected for the initial, infor-

In detail, the transformation of plants was carried out as follows.

2.1 Agrobacterium transformation

Leaf disks (Horsch et al., 1985) were used for transforming tobacco. For this purpose, leaves of about 2-3 cm in length from sterile shoot cultures were punched into disks of about 1 cm diameter and incubated together with a 24-hour cultivated Agrobacterium culture, after it had been taken up in 10 mM MgSO₄ and in 20 ml of MS medium (Murashige and Skoog, 1965), at 26°C for about 60 hours. The leaf disks were subsequently washed 3x in MS medium containing 1000 µg/ml claforan, and then laid out on MS plates which contained 0.5 µg/ml NAA (naphthylacetic acid), 0.2 µg/ml BAP (benzylaminopurine) and 2% sucrose ("callus induction medium") and the appropriate selective agent (e.g. kanamycin sulfate), and incubated at 26°C. After 2 weeks on each occasion, the leaf disks were transferred to fresh callus induction medium. Once small calluses could be distinguished, they were transferred to shoot induction medium, which corresponds to the

callus induction medium but contains 0.2 µg/ml NAA and 0.5 µg/ml BAP. Once the regenerated shoots had reached a length of 2-3 cm, they were transferred, for root induction, to MS medium which contained 1% sucrose but no hormones, and cultivated under sterile conditions at 24-26°C (12 hrs. of light (1000-3000 lux), 12 hrs. in the dark). After 4 to 6 weeks, the shoot cultures were transferred to fresh medium, grown into plants and subsequently cultivated under suitable conditions in a greenhouse.

For transforming potato plants, a further incubation of two days in the dark took place following incubation of the excised leaf disks in the Agrobacterium suspension. The leaf disks were then incubated for 8 days with 16-hour illumination on MS plates which contained 1.6% glucose, 5 mg/l NAA, 0.1 mg/l BAP, 500 mg/l claforan and the appropriate selective agent. The leaf disks were subsequently transferred to MS plates which contained 1.6% glucose, 2.0 mg/l zeatin riboside, 0.02 mg/l NAA, 0.02 mg/l GA3 (giberellic acid) and 500 mg/l claforan. Once the leaf disks had been incubated for 14 days, they were transferred to fresh plates. The first calluses appeared after about 6 to 7 weeks. Shoots which appeared were transferred to 250 ml glass flasks in which MS medium containing 3% sucrose and 0.5 mg/ml carbenicillin was present. The first roots appeared after about 14 days. The plants were subsequently cultivated under suitable

conditions in a greenhouse.

Tomatoes were transformed essentially in accordance with the abovedescribed process.

5 DNA (Dellaporta et al., 1983) and RNA (Goodall et al., 1990 and Chomczynski and Sacchi, 1987) were isolated from the resulting plants by standard processes. It was possible to determine the integrity and the number of the transferred genes by DNA analysis ("Southern blot"), and to determine the
10 amount of transcription by RNA analysis ("RNase protection" in accordance with Vankan and Filipowicz, 1988; Goodall and Filipowicz, 1989; Steinecke et al., 1992).

2.2 Protoplast isolation and PEG-mediated transformation

15 4-8 Week-old *Nicotiana tabacum* SR1 leaves from a sterile culture were cut off, cleaned with distilled H₂O, and placed in a 0.05% SDS solution in order to prevent bacterial contamination. The SDS was removed by washing twice with sterile H₂O, and the leaves
20 were then equilibrated for 10 min in K3 medium (500 mg/l claforan, 1 mg/l NAA, 0.2 mg/l kinetin). After removing the central rib, the leaves were cut into pieces of 1-2 cm² in size. In order to isolate the mesophyll protoplasts, the leaf pieces were
25 added to 30 ml of an enzyme solution containing cellulase and mazerzyme (1.5% cellulase,

0.5% mazeroyz in K3 medium) in a sterile 1 l flask and exposed to a slight vacuum for 10 min. Following subsequent incubation at 27°C for 16 h in the dark, the mixture was shaken for 30 min at 75 rpm. The

5 The protoplast suspension was then passed through steel sieves of 250 and 100 μ m pore width, and the filtrate was divided between 12 ml centrifuged tubes and centrifuged for 5 min at 650 rpm in a Hettich Universal 2S centrifuge. The lower phase together

10 with the sediment was sucked off using a 100 μ m glass capillary, the protoplasts were washed with 10 ml of K3 medium, and the medium was sucked off once again after the centrifugation (see above) had been repeated. Subsequently, the protoplasts were

15 taken up in W5 medium (154 mM NaCl, 125 mM $\text{CaCl}_2 \cdot x\text{H}_2\text{O}$, 5 mM glucose, 5 mM KCl, pH 5.6), counted in a Fuchs-Rosenthal counting chamber, and then left to stand for 30 min in W5 medium. The protoplasts were separated off by centrifuging at 650 rpm for 5 min, and the sediment was then suspended in MaMg solution

20 (450 mM mannitol, 15 mM MgCl_2 , 0.1% MES, pH 5.6), the concentration of the suspension being adjusted to $1-3 \times 10^6$ protoplasts/ml. It was then possible to transfect the protoplasts directly or to store them

25 for up to 4 h in a refrigerator.

For the transformation, the protoplasts were subjected to a 5-minutes heat shock at 45°C, while swirling occasionally, in a water bath, and then immediately cooled down to room temperature by being

placed for 45 s in ice/water. Subsequently, 0.35 ml volumes of protoplast suspension were aliquoted into 10 ml centrifuge tubes and 50 µl of the DNA solution were then added to each of the tubes. 0.35 ml of PEG solution (100 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 400 mM mannitol, 40% PEG 4000, pH 7-9) was added dropwise after 10 min, and the transfection sample was then transferred to Petri dishes after a further 20 min during which all the samples were swirled for 5 min. Following the dropwise addition of 4 ml of K3 medium, the protoplasts were cultivated at 27°C in the dark for 6-48 h. Following the incubation, the protoplasts were added to 12 ml centrifuge tubes, which were filled with sea water for washing and then centrifuged at 650 rpm for 3 min. The supernatant was removed down to 1 ml, and the protoplasts were then resuspended and transferred into Eppendorf tubes. After centrifuging at 13000 rpm (Biofuge) for 1 min and carefully removing the supernatant, the protoplasts were taken up in the relevant extraction buffers.

The DNA according to the invention were successfully introduced into protoplasts prepared by the above-described method.

3. Examples of the elevated resistance of transgenic plants according to the invention

3.1 Elevated resistance toward phytopathogenic fungi

The transgenic tobacco plants XL4, XL8, XL11, XL14, XL15 and XL19 which were listed under 2 (above), were selfed, and descendants of XL4 were chosen for the following test. The phytopathogenic fungus
5 *Botrytis cinerea* was used as the test pathogen.

(a) Test description

10 The tobacco plants were grown in a greenhouse at 23°C and 70-80% relative atmospheric humidity until the beginning of the experiment. Water and fertilizers are supplied according to requirement. For the inoculation, the leaves of the 6-8 week-old plants were sprayed until dripping wet with a spore suspension of the pathogen. Subsequently, the plants were
15 incubated under conditions which were favorable for the pathogen, i.e. at 20°C and 100% relative atmospheric humidity. After 4 days, the health status of the plants was ascertained on the basis of infected leaf area in percent. A distinction was made between
20 leaf stages (leaf 1 = oldest leaf, leaf 6 = youngest leaf).

(b) Test results

	Plants (47 in each case)	1-3 leaf	4-6 leaf	Average
5	<i>N. tabacum</i> (wild type); % infected leaf areas	22.4	24.0	23.2
10	Transgenic plants; % infected leaf area	14.3	17.1	15.7
15	Reduction in infestation in % (in accordance with Abbott)	36.3	29.0	32.5

3.2 Increased resistance toward viruses

20 The transgenic tobacco plants XL4, XL8, XL11, XL14, XL15 and XL19, which were listed under 2 (above), were selfed, and the homozygous descendants of XL4 (XL4.24) and XL8 (XL8.28) were chosen for the following test.

25 Various tospoviruses, and viruses of other families, were used as test pathogens.

(a) Test description

5 The tobacco plants were grown in a greenhouse at 23°C and 70-80% relative atmospheric humidity until the beginning of the experiment. Water and fertilizers are supplied according to requirement.

10 3 g of leaf material from primarily infected leaves exhibiting clear viral symptoms were harvested from tobacco plants (*Nicotiana rustica* L.) which had been infected 3 weeks previously with the TSWV isolate L3 (DSM No.: PV0182), and then homogenized in a mortar in 30 ml of standard buffer (0.1 M Na-K phosphate buffer, pH 7.0 containing 0.2% polyvinylpyrrolidone MW 10000 and 0.2% (w/v) Na_2SO_3). This material was used undiluted and in a 1:25 dilution in the standard buffer, and 0.1 ml was then in each case applied, mechanically by abrasion, per leaf to each of 3 leaves of a test plant which was about 8-10 weeks old. The test plants had previously been dusted with an abrasive (carborundum, 600 mesh), and were rinsed with mains water once inoculation had taken place.

25 The development of symptoms on the plants was assessed visually from the 5th day after inoculation over a time period of 14 days using a rating scheme. Symptoms appear both on the infected leaves and systemically on non-infected leaves.
Step 0 = no symptoms

Step 1 = mild symptoms, annular spots on the leaves
(necroses)

Step 2 = severe symptoms, vein yellowing, mosaics,
signs of die-back.

- 5 The rating values were used to elucidate the average
rating value, which was then related to the rating
values of the control plants.

- 10 In order further to quantify the resistance of the
plants, a triple-antibody sandwich (TAS) ELISA
process was used for determining the quantity of
TSWV antigen. For this, Greiner microtiter plates
(type 655001) were coated with 0.1 ml of polyclonal
antiserum (DSM No., AS-0105, 2 µg/ml) per well.
Using the standard ELISA buffer, pressed juices were
15 prepared in dilutions of 1:30 and 1:500 from test-
plant leaves which were primarily and secondarily
infected. 3 Wells of the plate were in each case
loaded with each sample (0.1 ml), and the plates
were then incubated at 4°C overnight. After the
plates had been washed three times with PBS-Tween,
20 0.1 ml of a 1:1000 dilution of the two monoclonal
antibodies 4F2 and 2B6 (Adam et al., 1991) was added
to each well, and the plates were incubated at 37°C
for 3 h. After the plates had been washed three
25 times with PBS-Tween, 0.1 ml of a 1:1000 dilution of
a rabbit anti-mouse IgG conjugate (DAK Diagnostica
GmbH, No.: D314) was added per well, and the plates
were then incubated at 37°C for 3 h. After the

plates had been washed three times with PBS-Tween, the substrate p-nitrophenyl phosphate (1 mg/ml) was added, and the extinction at 405 nm was measured in a photometer after 30 min and after 1 h. The extinction values were used to determine the average extinction value, which was then related to the control measurements. The results demonstrate that the transgenic plants are without symptoms whereas the control plants exhibit severe symptoms. In addition to this, it is found that the quantity of the TSWV antigen in the transgenic plants, detected by reaction with the monoclonal antibody (Mab 2B6), is markedly lower than in the control plants.

(b) Test result

Plants (in each case 4X10)	Severity of symptoms	Notes
<i>N. tabacum</i> (wild type)	2	The control plants only grow to half the height of the transgenic plants.
Transgenic XL 4.24	0	
Transgenic XL 8.28	0	

25 Biotest of XL 4.24 transgenic plants with various other tospovirus isolates

(a) Test description

Homozygous descendants of the transgenic plant XL4 (XL 4.24) were reared until they had reached the four-leaf stage.

- 5 The plants were randomly collected into groups of 20 and inoculated mechanically with homogenates from tobacco plants infected with various tospoviruses. The following virus isolates were used for the inoculation: TSWV-peanut = SA05 (DSM No. PV-0205);
- 10 INSV (DSM No. PV-0280); INSV (DSM No. PV-0281). The plants used for isolating the inoculum had been inoculated 10-14 days previously and were, on the basis of electron microscopic findings, free from contamination with other viruses. In order to pre-
- 15 pare the inoculum, the standard DSM inoculation buffer (DSM plant virus catalogue) was used, with the homogenisation being carried out using mortars and pestle and in the ratio 1:30 (g/ml). The inoculum was abraded, using sterile glass spatulas,
- 20 on to the test-plant leaves which had been dusted with carborundum (600 mesh). The two oldest secondary leaves of each test plant were inoculated. After the inoculation, the infected leaves were rinsed with mains water. The plants were
- 25 subsequently incubated under the abovementioned culture conditions and each day rated visually for symptoms. As controls, non-transformed *N. tabacum* plants were grown in the same way and inoculated

with the various isolates.

5 The final ratings of the experiment were made by two
observers at one and three weeks after the inocula-
tion; in cases where the assessments differed, that
which was less favorable was taken. The results are
summarized in Table 1. Following successful
infection, the TSWV-peanut (SA-05) isolate gave rise
to local necroses on the infected leaves and to
severe systemic symptoms, involving leaf deformation
10 and necroses on the newly formed, systemically
infected leaves, whereas the two INSV isolates only
gave rise to the formation of local necroses on the
inoculated leaves, without the occurrence of any
systemic spread to newly formed leaves. When carry-
15 ing out the rating in the case of TSWV-peanut, both
systemically infected test plants and those which
only exhibited local primary symptoms were taken to
be successfully infected, since experience showed
that in the latter case systemic symptoms were only
20 delayed and would appear with time. In the case of
the two INSV isolates, the appearance of local
primary symptoms was alone taken as evidence of
successful infection.

Table 1: Testing the transgenic tobacco line XL4 (XL4.24) for resistance toward TSWV-peanut and various other INSV isolates;

5	Isolate	Infected control plants following mechanical inoculation	Infected transgenic plants following mechanical inoculation	Features of the infected plants
10	TSWV-Peanut = SA05 (DSM No. PV-0205)	100%	4%	Severe systemic infection
15	INSV = (DSM No. PV-0280)	95%	55%	Local lesions on the inoculated leaves; no systemic infection
20	INSV (DSM No. PV-0281)	85%	35%	Local lesions on the inoculated leaves; no systemic infection

Biotest of XL 4.24 transgenic plants using virus isolates which do not belong to the bunyavirus family.

(a) Test description

25 Homozygous descendants of the transgenic plant XL4 (XL 4.24) were raised until they had reached the four-leaf stage.

30 The plants were randomly collected into groups of 20 and inoculated mechanically with homogenates from tobacco plants infected with various viruses. The following virus isolates were used for the

inoculation: TMV (DSM No. PV-0055) and tobacco
rattle virus (DSM No. PV-0043). The plants used for
isolating the inoculum had been inoculated 10-14
days previously and were, on the basis of electron
microscopic findings, free from contamination with
other viruses. In order to prepare the inoculum, the
standard DSM inoculation buffer (DSM plant virus
catalogue) was used, with the homogenization being
carried out using mortars and pestle and in the
ratio 1:30 (g/ml). The inoculum was abraded, using
sterile glass spatulas, onto the test-plant leaves,
which had been dusted with carborundum (600 mesh).
The two oldest secondary leaves of each test plant
were inoculated. After the inoculation, the infected
leaves were rinsed with mains water. The plants were
subsequently incubated under the abovementioned
culture conditions and each day rated visually for
symptoms. As controls, non-transformed *N. tabacum*
plants were grown in the same way and inoculated
with the various isolates.

The final ratings of the experiment were made by two
observers at one and three weeks after the
inoculation; in cases where the assessments
differed, the less favorable was taken. While infec-
tion with both the viruses gave rise in the control
plants to local necroses on the primarily infected
leaves, that with TRV also gave rise to severe
systemic symptoms. In the case of TMV, significantly
fewer local lesions were observed in the transgenic

5 XL4.24 plants. In the case of the tobacco rattle virus, 14% of the controls only had necroses on the primarily infected leaves, whereas 86% also exhibited systemic systems. By contrast, 28% of the transgenic plants were completely free of infection, 36% merely had primary necroses, and only 36% had systemic systems as well.

3.3 Elevated resistance toward phytopathogenic nematodes

(a) Test description

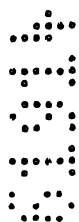
10 The transgenic tobacco plants (XL4) were grown in a greenhouse at 23°C and 70-80% relative atmospheric humidity until the beginning of the experiment. After pricking out, the plants were grown on in soil in order to ensure a particularly well-developed

15 root system. Water and fertilizers were supplied according to requirement. Plants which were about 15 cm in height were inoculated by pipetting viable, freshly hatched larvae of *Meloidogyne incognita* (LII) uniformly on to the soil surface at the rate of

20 about 300 larvae per 100 ml of soil volume. Rating took place 4 weeks after inoculation by determining the number of galls on each root system.

25 In comparison to the wild type plants, the transgenic plants exhibit increased resistance toward nematodes.

In the present text, % values relate to percentages by weight, unless otherwise indicated, and dilutions to parts by volume (e.g. 1:25), unless otherwise indicated.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

5 (A) NAME: Bayer AG
(B) STREET: Bayerwerk
(C) CITY: Leverkusen
(E) COUNTRY: Germany
(F) POSTAL CODE: 5090
10 (G) TELEPHONE: 0214/30 61285
(H) TELEFAX: 0214/30 3482
(I) TELEX: 85 101-265 by d

(ii) TITLE OF APPLICATION: deoxyribonucleic acids

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:

15 (A) MEDIUM TYPE: floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0,
Version #1.25 (EPA)

20 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1040 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

5

(A) NAME/KEY: CDS

(E) LENGTH: 36..989

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

AAAATATAAA AACTCAACAC AACATACAA ATTTT ATG CAA TCA AAT CAA TCT	53
Met Gln Ser Asn Gln Ser	
1 5	
CAA GCT ATC AAA ATT TTT CGA ATC TCA CTT GAA AGA TCA AAA ATC AAC	101
Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu Glu Arg Ser Lys Ile Asn	
10 15 20	
AAA GAA AAT TCC TTA ATT TCT TAA CTA AAT TTA CTG CAA GTC AAG ATG	149
Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn Leu Leu Gln Val Lys Met	
25 30 35	
TCG AGA ATT CGA GCT CGG TAC CCA CCC GAT CCT CTA GAG TCG CAT ATA	197
Ser Arg Ile Arg Ala Arg Tyr Pro Pro Asp Pro Leu Glu Ser His Ile	
40 45 50	
ACA ACT TCT ACA ATC ATC ATG TCT AAG GTT AAG CTC ACT AAG GAA AGC	245
Thr Thr Ser Thr Ile Ile Met Ser Lys Val Lys Leu Thr Lys Glu Ser	
55 60 65 70	

ATT GTT GCT TTG TTG ACA CAA GGC AAA GAC CTT GAG TTT GAG GAA GAT	293
Ile Val Ala Leu Leu Thr Gln Gly Lys Asp Leu Glu Phe Glu Glu Asp	
75 80 85	
CAG AAT CTG GTA GCA TTC AAC TTC AAG ACT TTT TGT CTA GAA AAC CTC	341
Gln Asn Leu Val Ala Phe Asn Phe Lys Thr Phe Cys Leu Glu Asn Leu	
90 95 100	
GAC CAG ATC AAG AAG ATG AGC GTT ATT TCA TGT CTG ACG TTC CTG AAG	389
Asp Gln Ile Lys Lys Met Ser Val Ile Ser Cys Leu Thr Phe Leu Lys	
105 110 115	
AAT CGT CAG AGT ATA ATG AAG GTT ATT AAA CAA AGT GAT TTT ACT TTT	437
Asn Arg Gln Ser Ile Met Lys Val Ile Lys Gln Ser Asp Phe Thr Phe	
120 125 130	
GGT AAA ATT ACC ATA AAG AAA ACT TCA GAC AGG ATT GGA GCC ACT GAC	485
Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp Arg Ile Gly Ala Thr Asp	
135 140 145 150	
ATG ACC TTC AGA AGG CTT GAT AGC TTG ATC AGG GTC AGG CTT GTA GAG	533
Met Thr Phe Arg Arg Leu Asp Ser Leu Ile Arg Val Arg Leu Val Glu	
155 160 165	
GAA ACC GGG AAT TCT GAG AAT CTC AAT ACT ATC AAA TCT AAG ATT GCT	581
Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr Ile Lys Ser Lys Ile Ala	
170 175 180	
TCC CAC CCT TTG ATT CAA GCC TAT GGA TTA CCT CTT GAT GAT GCA AAG	629
Ser His Pro Leu Ile Gln Ala Tyr Gly Leu Pro Leu Asp Asp Ala Lys	
185 190 195	

TCT GTG AGG CTT GCC ATA ATG CTG GGA GGT AGC TTA CCT CTT ATT GCT Ser Val Arg Leu Ala Ile Met Leu Gly Gly Ser Leu Pro Leu Ile Ala 200 205 210	677
TCA GTT GAT AGC TTT GAG ATG ATC AGT GTT GTC TTG GCT ATA TAT CAG Ser Val Asp Ser Phe Glu Met Ile Ser Val Val Leu Ala Ile Tyr Gln 215 220 225 230	725
GAT GCA AAA TAC AAA GAC CTC GGG ATT GAC CCA AAG AAG TAT GAC ACC Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp Pro Lys Lys Tyr Asp Thr 235 240 245	773
AAG GAA GCC TTA GGA AAA GTT TGC ACT GTG CTG AAA AGC AAA GCA TTT Lys Glu Ala Leu Gly Lys Val Cys Thr Val Leu Lys Ser Lys Ala Phe 250 255 260	821
GAA ATG AAT GAA GAT CAG GTG AAG AAA GGA AAA GAG TAT GCT GCT ATA Glu Met Asn Glu Asp Gln Val Lys Lys Gly Lys Glu Tyr Ala Ala Ile 265 270 275	869
CTC AGC TCC AGC AAT CCT AAT GCT AAA GGA AGT ATT GCT ATG GAA CAT Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly Ser Ile Ala Met Glu His 280 285 290	917
TAC AGT GAA ACT CTT AAC AAG TTC TAT GAA ATG TTT GGG GTT AAA AAA Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu Met Phe Gly Val Lys Lys 295 300 305 310	965
CAG GCA AAA CTC ACA GAA CTT GCT TAAAAGCAGT TGTAAGTTAA ATTATGGAAA Gln Ala Lys Leu Thr Glu Leu Ala 315	1019
AGTCTACAAA TATATAAGC T	1040

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 318 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

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

Met Gln Ser Asn Gln Ser Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu
 1 5 10 15
 Glu Arg Ser Lys Ile Asn Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn
 20 25 30
 Leu Leu Gln Val Lys Met Ser Arg Ile Arg Ala Arg Tyr Pro Pro Asp
 35 40 45
 Pro Leu Glu Ser His Ile Thr Thr Ser Thr Ile Ile Met Ser Lys Val
 50 55 60
 Lys Leu Thr Lys Glu Ser Ile Val Ala Leu Leu Thr Gln Gly Lys Asp
 65 70 75 80
 Leu Glu Phe Glu Glu Asp Gln Asn Leu Val Ala Phe Asn Phe Lys Thr
 85 90 95
 Phe Cys Leu Glu Asn Leu Asp Gln Ile Lys Lys Met Ser Val Ile Ser
 100 105 110
 Cys Leu Thr Phe Leu Lys Asn Arg Gln Ser Ile Met Lys Val Ile Lys
 115 120 125
 Gln Ser Asp Phe Thr Phe Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp
 130 135 140

Arg Ile Gly Ala Thr Asp Met Thr Phe Arg Arg Leu Asp Ser Leu Ile
 145 150 155 160
 Arg Val Arg Leu Val Glu Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr
 165 170 175
 Ile Lys Ser Lys Ile Ala Ser His Pro Leu Ile Gln Ala Tyr Gly Leu
 180 185 190
 Pro Leu Asp Asp Ala Lys Ser Val Arg Leu Ala Ile Met Leu Gly Gly
 195 200 205
 Ser Leu Pro Leu Ile Ala Ser Val Asp Ser Phe Glu Met Ile Ser Val
 210 215 220
 Val Leu Ala Ile Tyr Gln Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp
 225 230 235 240
 Pro Lys Lys Tyr Asp Thr Lys Glu Ala Leu Gly Lys Val Cys Thr Val
 245 250 255
 Leu Lys Ser Lys Ala Phe Glu Met Asn Glu Asp Gln Val Lys Lys Gly
 260 265 270

Lys Glu Tyr Ala Ala Ile Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly
 275 280 285
 Ser Ile Ala Met Glu His Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu
 290 295 300
 Met Phe Gly Val Lys Lys Gln Ala Lys Leu Thr Glu Leu Ala
 305 310 315

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 439 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

AAGCTTGCAT GCCTGCAGGT CAACATGGTG GAGCAGGACA CTCTCGTCTA CTCCAAGAAT	60
ATCAAAGATA CAGTCTCAGA AGACCAAAGG GCTATTGAGA CTTTTCACAA AAGGGTAATA	120
TCGGGAAGACC TCCTCGGATT CCATTGCCCA GCTATCTGTC ACTTGATCRA AAGGACAC JA	180
GAAAAGGAAG GTGGCACCTA CAATGCCAT CATTGCGATA AAGGAAAGGC TATCGTTCAA	240
GAATGCCTCT GCGACAGTG GTCCCAAAGA TGGACCCCA CCCACGAGGA GCATCGTGGA	300
AAAAGAAGAC GTTCCAACCA CTCTTCAAA GCAAGTGGAT TGATGTGATA TCTCCACTGA	360
CGTAAGGGAT GACGCACAAT CCACTATCC TTCGCAAGAC CCTTCCTCTA TATAAGGAAG	420
TTCAATTCAT TTGGAGAGG	439

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 226 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

CGCAAAATC ACCAGTCTCT CTCTACAAAT CTATCTCTCT CTATTTCTCT CCAGAATAAT	60
GTGTGAGTAG TTCCAGATA AGGGAATTAG GGTTCCTTATA GGGTTTCGCT CATGTGTTGA	120
GCATATAAGA ARCCCTTAGT ATGTATTTGT ATTTGTAAAA TACTTCTATC AATAAAATTT	180
CTAATTCCTA AAACCAAAAT CCAGTGACCT GCAGGCATGC AAGCTT	226

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1722 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

(A) NAME/KEY: CDS
(B) LENGTH: 475..1428

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

AAGC ATGCAT GCCTGCAGGT CAACATGGTG GAGCAGGACA CTCTCGTCTA CTCGAAGAAT 60
 ATCAAAGATA CAGTCTCAGA AGACCAAGG GCTATTGAGA CTTTTCACCA AAGGGTAATA 120
 TCGGGAAACC TCCTCGGATT CCATTGCCCA GCTATCTGTC ACTTGATCAA AAGGACAGTA 180
 GAAAAGGAAG GTGGCACCTA CAAATGCCAT CATTGCGATA AAGGAAAGGC TATCCTTCAA 240
 GAATGCCTCT GCCGACAGTG GTCCCAAGA TGGACCCCA CCCACGAGGA GCATCTTCA 300
 AAAAGAAGAC GTTCCAACCA CGTCTTCAA GCAAGTGGAT TGATGTGATA TCTCCACTGA 360
 CGTAAGGGAT GACGCACAAT CCCACTATCC TTCGCAAGAC CCTTCCTCTA TATAAGGAAG 420
 TTCATTTTCA TTGGAGAGGA AAATATATPA ACTCAACACA ACATACAAAA TTTT ATG 477
 Met
 1

CAA TCA AAT CAA TCT CAA GCT ATC AAA ATT TTT CGA ATC TCA CTT GAA 525
 Gln Ser Asn Gln Ser Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu Glu
 5 10 15
 AGA TCA AAA ATC AAC AAA GAA AAT TCC TTA ATT TCT TTA CTA AAT TTA 573
 Arg Ser Lys Ile Asn Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn Leu
 20 25 30
 CTG CAA GTC AAG ATG TCG AGA ATT CGA GCT CGG TAC CCA CCC GAT CCT 621
 Leu Gln Val Lys Met Ser Arg Ile Arg Ala Arg Tyr Pro Pro Asp Pro
 35 40 45
 CTA GAG TCG CAT ATA ACA ACT TCT ACA ATC ATC ATG TCT AAG GTT AAG 669
 Leu Glu Ser His Ile Thr Ser Thr Ile Ile Met Ser Lys Val Lys
 50 55 60 65
 CTC ACT AAG GAA AGC ATT GTT GCT TTG TTG ACA CAA GGC AAA GAA CTT 717
 Leu Thr Lys Glu Ser Ile Val Ala Leu Leu Thr Gln Gly Lys Asp Leu
 70 75 80
 GAG TTT GAG GAA GAT CAG AAT CTG GTA GCA TTC AAC TTC AAG ACT TTT 765
 Glu Phe Glu Glu Asp Gln Asn Leu Val Ala Phe Asn Phe Lys Thr Phe
 85 90 95
 TGT CTA GAA AAC CTC GAC CAG ATC AAG AAG ATG AGC GTT ATT TCA TGT 813
 Cys Leu Glu Asn Leu Asp Gln Ile Lys Lys Met Ser Val Ile Ser Cys
 100 105 110

CTG ACG TTC CTG AAG AAT CGT CAG AGT ATA ATG AAG GTT ATT AAA CAA Leu Thr Phe Leu Lys Asn Arg Gln Ser Ile Met Lys Val Ile Lys Gln 115 120 125	861
AGT GAT TTT ACT TTT GGT AAA ATT ACC ATA AAG AAA ACT TCA GAC AGG Ser Asp Phe Thr Phe Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp Arg 130 135 140 145	909
ATT GGA GCC ACT GAC ATG ACC TTC AGA AGG CTT GAT AGC TTG ATC AGG Ile Gly Ala Thr Asp Met Thr Phe Arg Arg Leu Asp Ser Leu Ile Arg 150 155 160	957
GTC AGG CTT GTA GAG GAA ACC GGG AAT TCT GAG AAT CTC AAT ACT ATC Val Arg Leu Val Glu Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr Ile 165 170 175	1005
AAA TCT AAG ATT GCT TCC CAC CCT TTG ATT CAA GCC TAT GGA TTA CCT Lys Ser Lys Ile Ala Ser His Pro Leu Ile Gln Ala Tyr Gly Leu Pro 180 185 190	1053
CTT GAT GAT GCA AAG TCT GTG AGG CTT GCC ATA ATG CTG GGA GGT AGC Leu Asp Asp Ala Lys Ser Val Arg Leu Ala Ile Met Leu Gly Gly Ser 195 200 205	1101
TTA CCT CTT ATT GCT TCA GTT GAT AGC TTT GAG ATG ATC AGT GTT GTC Leu Pro Leu Ile Ala Ser Val Asp Ser Phe Glu Met Ile Ser Val Val 210 215 220 225	1149
TTG GCT ATA TAT CAG GAT GCA AAA TAC AAA GAC CTC GGG ATT GAC CCA Leu Ala Ile Tyr Gln Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp Pro 230 235 240	1197
AAG AAG TAT GAC ACC AAG GAA GCC TTA GGA AAA GTT TGC ACT GTG CTG Lys Lys Tyr Asp Thr Lys Glu Ala Leu Gly Lys Val Cys Thr Val Leu 245 250 255	1245
AAA AGC AAA GCA TTT GAA ATG AAT GAA GAT CAG GTG AAG AAA GGA AAA Lys Ser Lys Ala Phe Glu Met Asn Glu Asp Gln Val Lys Lys Gly Lys 260 265 270	1293
GAG TAT GCT GCT ATA CTC AGC TCC AGC AAT CCT AAT GCT AAA GGA AGT Glu Tyr Ala Ala Ile Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly Ser 275 280 285	1341
ATT GCT ATG GAA CAT TAC AGT GAA ACT CTT AAC AAG TTC TAT GAA ATG Ile Ala Met Glu His Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu Met 290 295 300 305	1389
TTT GGG GTT AAA AAA CAG GCA AAA CTC ACA GAA CTT GCT TAAAAGCAGT Phe Gly Val Lys Lys Gln Ala Lys Leu Thr Glu Leu Ala 310 315	1438

TGTAAGTTAA ATTATGGAAG AGTCTACAAA TATATAAAGC TGGGGATCCT CTAGAGTCCG	1498
CAAAAATCAC CAGTCTCTCT CTACAAATCT ATCTCTCTCT ATTTTCTCC AGAATAATGT	1558
GTGAGTAGTT CCCAGATAAG GGAATTAGGG TTCTTATAGG GTTTCGCTCA TGTGTTGAGC	1618
ATATAAGAAA CCCTTAGTAT GTATTGTAT TTGTAAAATA CTTCTATCAA TAAATTCT	1678
AATTCCTAAA ACCAAAATCC AGTGACCTGC AGGCATGCAA GCTT	1722

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 318 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

5

Met Gln Ser Asn Gln Ser Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu
 1 5 10 15
 Glu Arg Ser Lys Ile Asn Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn
 20 25 30
 Leu Leu Gln Val Lys Met Ser Arg Ile Arg Ala Arg Tyr Pro Pro Asp
 35 40 45
 Pro Leu Glu Ser His Ile Thr Thr Ser Thr Ile Ile Met Ser Lys Val
 50 55 60
 Lys Leu Thr Lys Glu Ser Ile Val Ala Leu Leu Thr Gln Gly Lys Asp
 65 70 75 80
 Leu Glu Phe Glu Glu Asp Gln Asn Leu Val Ala Phe Asn Phe Lys Thr
 85 90 95
 Phe Cys Leu Glu Asn Leu Asp Gln Ile Lys Lys Met Ser Val Ile Ser
 100 105 110
 Cys Leu Thr Phe Leu Lys Asn Arg Gln Ser Ile Met Lys Val Ile Lys
 115 120 125
 Gln Ser Asp Phe Thr Phe Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp
 130 135 140
 Arg Ile Gly Ala Thr Asp Met Thr Phe Arg Arg Leu Asp Ser Leu Ile
 145 150 155 160

Arg Val Arg Leu Val Glu Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr
 165 170 175
 Ile Lys Ser Lys Ile Ala Ser His Pro Leu Ile Gln Ala Tyr Gly Leu
 180 185 190
 Pro Leu Asp Asp Ala Lys Ser Val Arg Leu Ala Ile Met Leu Gly Gly
 195 200 205
 Ser Leu Pro Leu Ile Ala Ser Val Asp Ser Phe Glu Met Ile Ser Val
 210 215 220
 Val Leu Ala Ile Tyr Gln Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp
 225 230 235 240
 Pro Lys Lys Tyr Asp Thr Lys Glu Ala Leu Gly Lys Val Cys Thr Val
 245 250 255
 Leu Lys Ser Lys Ala Phe Glu Met Asn Glu Asp Gln Val Lys Lys Gly
 260 265 270
 Lys Glu Tyr Ala Ala Ile Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly
 275 280 285
 Ser Ile Ala Met Glu His Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu
 290 295 300
 Met Phe Gly Val Lys Lys Gln Ala Lys Leu Thr Glu Leu Ala
 305 310 315

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 152 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LENGTH: 36..152

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

```
AAAATATAAA AACTCAACAC AACATACAAA ATTTT ATG CAA TCA AAT CAA TCT      53
                               Met Gln Ser Asn Gln Ser
                               1           5

CAA GCT ATC AAA ATT TTT CGA ATC TCA CTT GAA AGA TCA AAA ATC AAC      101
Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu Glu Arg Ser Lys Ile Asn
          10           15           20

AAA GAA AAT TCC TTA ATT TCT TTA CTA AAT TTA CTG CAA GTC AAG ATG      149
Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn Leu Leu Gln Val Lys Met
          25           30           35

TCG      152
Ser
```

5 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Gln Ser Asn Gln Ser Gln Ala Ile Lys Ile Phe Arg Ile Ser Leu
  1           5           10           15

Glu Arg Ser Lys Ile Asn Lys Glu Asn Ser Leu Ile Ser Leu Leu Asn
          20           25           30

Leu Leu Gln Val Lys Met Ser
          35
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

- (A) NAME/KEY: CDS
- (B) LENGTH: 1..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGA ATT CGA GCT CGG TAC CCA CCC GAT CCT CTA GAG TC
Arg Ile Arg Ala Arg Tyr Pro Pro Asp Pro Leu Glu
1 5 10

38

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Arg Ile Arg Ala Arg Tyr Pro Pro Asp Pro Leu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 850 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURES:

- 10 (A) NAME/KEY: CDS
(B) LENGTH: 2..799

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

G	CAT	ATA	ACA	ACT	TCT	ACA	ATC	ATC	ATG	TCT	AAG	GTT	AAG	CTC	ACT	46
	His	Ile	Thr	Thr	Ser	Thr	Ile	Ile	Met	Ser	Lys	Val	Lys	Leu	Thr	
	1				5				10					15		
AAG	GAA	AGC	ATT	GTT	GCT	TTG	TTG	ACA	CAA	GGC	AAA	GAC	CTT	GAG	TTT	94
Lys	Glu	Ser	Ile	Val	Ala	Leu	Leu	Thr	Gln	Gly	Lys	Asp	Leu	Glu	Phe	
				20					25					30		

GAG GAA GAT CAG AAT CTG GTA GCA TTC AAC TTC AAG ACT TTT TGT CTA Glu Glu Asp Gln Asn Leu Val Ala Phe Asn Phe Lys Thr Phe Cys Leu 35 40 45	142
GAA AAC CTC GAC CAG ATC AAG AAG ATG AGC GTT ATT TCA TGT CTG ACG Glu Asn Leu Asp Gln Ile Lys Lys Met Ser Val Ile Ser Cys Leu Thr 50 55 60	190
TTC CTG AAG AAT CGT CAG AGT ATA ATG AAG GTT ATT AAA CAA AGT GAT Phe Leu Lys Asn Arg Gln Ser Ile Met Lys Val Ile Lys Gln Ser Asp 65 70 75	238
TTT ACT TTT GGT AAA ATT ACC ATA AAG AAA ACT TCA GAC AGG ATT GGA Phe Thr Phe Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp Arg Ile Gly 80 85 90 95	286
GCC ACT GAC ATG ACC TTC AGA AGG CTT GAT AGC TTG ATC AGG GTC AGG Ala Thr Asp Met Thr Phe Arg Arg Leu Asp Ser Leu Ile Arg Val Arg 100 105 110	334
CTT GTA GAG GAA ACC GGG AAT TCT GAG AAT CTC AAT ACT ATC AAA TCT Leu Val Glu Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr Ile Lys Ser 115 120 125	382
AAG ATT GCT TCC CAC CCT TTG ATT CAA GCC TAT GGA TTA CCT CTT GAT Lys Ile Ala Ser His Pro Leu Ile Gln Ala Tyr Gly Leu Pro Leu Asp 130 135 140	430
GAT GCA AAG TCT GTG AGG CTT GCC ATA ATG CTG GGA GGT AGC TTA CCT Asp Ala Lys Ser Val Arg Leu Ala Ile Met Leu Gly Gly Ser Leu Pro 145 150 155	478

CTT ATT GCT TCA GTT GAT AGC TTT GAG ATG ATC AGT GTT GTC TTG GCT Leu Ile Ala Ser Val Asp Ser Phe Glu Met Ile Ser Val Val Leu Ala 160 165 170 175	526
ATA TAT CAG GAT GCA AAA TAC AAA GAC CTC GGG ATT GAC CCA AAG AAG Ile Tyr Gln Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp Pro Lys Lys 180 185 190	574
TAT GAC ACC AAG GAA GCC TTA GGA AAA GGT TGC ACT GTG CTG AAA AGC Tyr Asp Thr Lys Glu Ala Leu Gly Lys Val Cys Thr Val Leu Lys Ser 195 200 205	622
AAA GCA TTT GAA ATG AAT GAA GAT CAG GTG AAG AAA GGA AAA GAG TAT Lys Ala Phe Glu Met Asn Glu Asp Gln Val Lys Lys Gly Lys Glu Tyr 210 215 220	670
GCT GCT ATA CTC AGC TCC AGC AAT CCT AAT GCT AAA GGA AGT ATT GCT Ala Ala Ile Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly Ser Ile Ala 225 230 235	718
ATG GAA CAT TAC AGT GAA ACT CTT AAC AAG TTC TAT GAA ATG TTT GGG Met Glu His Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu Met Phe Gly 240 245 250 255	766
GTT AAA AAA CAG GCA AAA CTC ACA GAA CTT GCT TAAAAGCAGT TGTAAGTTAA Val Lys Lys Gln Ala Lys Leu Thr Glu Leu Ala 260 265	819
ATTATGGAAA AGTCTACAAA TATATAAAGC T	850

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 266 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Ile Thr Thr Ser Thr Ile Ile Met Ser Lys Val Lys Leu Thr Lys
 1 5 10 15
 Glu Ser Ile Val Ala Leu Leu Thr Gln Gly Lys Asp Leu Glu Phe Glu
 20 25 30
 Glu Asp Gln Asn Leu Val Ala Phe Asn Phe Lys Thr Phe Cys Leu Glu
 35 40 45
 Asn Leu Asp Gln Ile Lys Lys Met Ser Val Ile Ser Cys Leu Thr Phe
 50 55 60
 Leu Lys Asn Arg Gln Ser Ile Met Lys Val Ile Lys Gln Ser Asp Phe
 65 70 75 80
 Thr Phe Gly Lys Ile Thr Ile Lys Lys Thr Ser Asp Arg Ile Gly Ala
 85 90 95
 Thr Asp Met Thr Phe Arg Arg Leu Asp Ser Leu Ile Arg Val Arg Leu
 100 105 110
 Val Glu Glu Thr Gly Asn Ser Glu Asn Leu Asn Thr Ile Lys Ser Lys
 115 120 125

Ile Ala Ser His Pro Leu Ile Gln Ala Tyr Gly Leu Pro Leu Asp Asp
 130 135 140
 Ala Lys Ser Val Arg Leu Ala Ile Met Leu Gly Gly Ser Leu Pro Leu
 145 150 155 160
 Ile Ala Ser Val Asp Ser Phe Glu Met Ile Ser Val Val Leu Ala Ile
 165 170 175
 Tyr Gln Asp Ala Lys Tyr Lys Asp Leu Gly Ile Asp Pro Lys Lys Tyr
 180 185 190
 Asp Thr Lys Glu Ala Leu Gly Lys Val Cys Thr Val Leu Lys Ser Lys
 195 200 205
 Ala Phe Glu Met Asn Glu Asp Gln Val Lys Lys Gly Lys Glu Tyr Ala
 210 215 220
 Ala Ile Leu Ser Ser Ser Asn Pro Asn Ala Lys Gly Ser Ile Ala Met
 225 230 235 240
 Glu His Tyr Ser Glu Thr Leu Asn Lys Phe Tyr Glu Met Phe Gly Val
 245 250 255
 Lys Lys Gln Ala Lys Leu Thr Glu Leu Ala
 260 265

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGGGATCCTC TAGAGTC

17

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TGTGTTGAGT TTTTATATTT TCCTCTCCAA ATGAAA

36

(2) INFORMATION FOR SEQ ID NO: 15:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AGAGG

5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AAAATAT

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTCAGTGGCT CCAATCCTGT CTGAAG

26

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TGTCGAGAAT TCGAGCICGG TAC

23



The Claims defining the invention are as follows:

1. Recombinant deoxyribonucleic acid (DNA), which is characterized in that they consist of a combination of the following constituents, or contain these constituents:
 - (a) a double-stranded cDNA fragment which is derived from the RNA of the plum pox virus (PPV) ("fragment A"), and
 - (b) a double-stranded cDNA fragment which is derived from the S RNA of the N structural gene of the tomato spotted wilt virus (TSWV) ("fragment B"), it being possible for this combination also to possess, in addition to further DNA fragments which may optionally be present, a promoter which is active in plant cells and which regulates transcription of the recombinant DNA fragments.
2. DNA according to claim 1, where fragment B is situated after (i.e. in the 3' direction) fragment A.
3. DNA according to claim 1, where fragment A is a DNA having the sequence listed in SEQ ID No:7, or is a DNA having the same effect.
4. DNA according to claim 1, where fragment B is a DNA having the sequence listed in SEQ ID No:11, or is a

DNA having the same effect.

5. DNA according to claim 1, which contains a combination of the DNA fragments which are listed in SEQ ID No:7 and SEQ ID No:11, or a DNA combination having the same effect, or consists of such a combination.
6. DNA according to claim 1, which contains a promoter which is active in plants.
7. DNA according to claim 1, which contains as the promoter, a DNA having the CaMV 35S promoter sequence, or a DNA having the same effect.
8. DNA according to claim 1, in which the fragments A and B are linked via a synthetic cDNA fragment ("fragment C") having up to 134 base pairs, which latter fragment connects the open reading frame beginning in fragment A to fragment B.
9. DNA according to claim 1, in which the A and B cDNA fragments are connected via the synthetic DNA fragment having the sequence according to SEQ ID No:9, or via a DNA having the same effect.
10. DNA according to claim 1, which contains, following fragment B, a double-stranded DNA fragment ("fragment D") having 1 to 30 base pairs, or a DNA having the same effect.

11. DNA according to claim 1, in which is contained, following fragment B, a double-stranded DNA fragment according to SEQ ID No:13, or a DNA having the same effect.
- 5 12. DNA according to claim 1, which contains a 3' untranslated DNA which contains a transcription termination sequence and a polyadenylation site and which either follows fragment B directly or is connected to fragment B via a double-stranded DNA fragment (fragment D).
- 10 13. DNA according to claim 1, which contains a 3' untranslated DNA which either follows fragment B directly or is connected to fragment B via a double-stranded DNA fragment (fragment D), the 3' untranslated DNA possessing the sequence according to SEQ ID No:4.
- 15 14. DNA according to claim 1, which consists of a DNA having the sequence listed in SEQ ID No:1, or of a DNA having the same effect, or contains this sequence.
- 20 15. DNA according to claim 1, which consists of a DNA having the sequence listed in SEQ ID No:5, or of a DNA having the same effect, or contains this sequence.
16. Viral and prokaryotic DNA, which contains a DNA according to claim 1.



17. Vegetable DNA, which contains a DNA according to claim 1.
18. Vectors, containing a DNA according to claim 1.
19. Host organisms, containing a DNA according to claim 1.
20. Use of the recombinant DNA according to claim 1 for producing plants, plant parts and plant cells (including multiplication material) possessing elevated resistance toward pernicious organisms or plant diseases.
21. Process for preparing transgenic plant cells and plants, characterized in that the recombinant DNA according to claim 1 is inserted, by customary methods, into the genome of the plant cells or plants, plant cells are optionally regenerated to give plants, the plants are optionally multiplied, and, optionally for producing further plants, the genome of the transgenic plants is combined with the genome of further plants, and the descendants are multiplied.
22. Transgenic plant cells, plants and their parts, as well as multiplication material, which contains the recombinant DNA according to claim 1.
23. Transgenic plant cells, plants and their parts, as

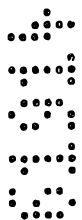
well as multiplication material, which contain a
protein according to SEQ ID No:2 or 6.

DATED this 5th day of May 1994,

BAYER AKTIENGESELLSCHAFT

By Its Patent Attorney

DAVIES COLLISON CAVE



Deoxyribonucleic acids

A b s t r a c t

The present invention relates to novel, recombinant deoxyribonucleic acids (DNA), to vectors and host organisms which contain them, and to transgenic plants which contain the recombinant DNA and possess an elevated resistance toward pernicious organisms and plant diseases, where the recombinant deoxyribonucleic acids (DNA) are characterized in that they consist of a combination of the following components, or components possessing a DNA which in each case has the same effect, or contain these constituents:

- (a) a double-stranded cDNA fragment which is derived from the RNA of the plum pox virus (PPV) ("fragment A"), and
- (b) a double-stranded cDNA fragment which is derived from the S RNA of the tomato spotted wilt virus (TSWV) ("fragment B"), it being possible for these combinations also to possess, in addition to further DNA fragments which may optionally be present, a promoter which is active in plant cells.