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NOTICE OF ENTITLEMENT

We, Sandoz Ltd., the applicant/Nominated Person in respect of Application No. 70707/94 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventor by assignment.

The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person made the application listed in the declaration under Article 8 of the PCT.

DATED this TWENTY NINTH day of NOVEMBER 1995



a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

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(57) Claim

1. A DNA construct capable of encoding directly or indirectly for a minus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant such that at least one protein, polypeptide or peptide eliciting a natural plant defence mechanism against invading pathogens is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus, which construct is under expression control of a promoter and a terminator capable of functioning in plants.
2. A recombinant DNA construct capable of encoding for a plus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant and replicating as a result of such interaction a plus sense RNA molecule which is capable of encoding for at least one protein, polypeptide or peptide eliciting a natural plant defence mechanism against invading pathogens, which construct is under expression control of a promoter and a terminator capable of functioning in plants.
3. A construct according to claim 1 comprising SEQ ID NO:1.

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(57) Abstract DNA constructs encoding an RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant such that either an eliciting element or a plus sense RNA is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus, whereby any produced plus sense RNA molecule is capable of encoding for an eliciting element, plants containing such constructs and processes for obtaining such plants.			

VIRUS RESISTANT PLANTS

BACKGROUND

The present invention relates to pathogen resistant plants (and in particular to pathogen resistant plants wherein pathogen resistance is triggered in response to invading pathogens such as viruses,) DNA constructs for use in such plants and methods of introducing virus induced resistance into plants.

Viral infections in plants are frequently responsible for detrimental effects in growth, undesirable morphological changes, decreased yield and the like. Such infections often result in a higher susceptibility to infection in infected plants to other plant pathogens and plant pests.

Virus particles generally comprise a relatively small amount of genetic material (single or double stranded RNA or DNA) protected by a protein or proteins which in some viral types can also be surrounded with host-derived lipid membranes, yielding infectious particles. Viruses are dependent on host cells for multiplication and may therefore be regarded as intracellular parasites.

Plants have evolved a number of defensive mechanisms to limit the effects of viral infection. For example, so-called horizontal or partial resistances which are polygenic in nature and so-called vertical resistances which are monogenic in nature.

Horizontal resistance is difficult to introduce successfully into plants in breeding programs, however, vertical resistance can be bred into plants relatively easily within plant breeding programs. Genes coding for virus resistance can act constitutively in a passive sense, ie without a requirement for inducing gene expression. Constitutively expressed virus resistances include as modes of action non-host resistances,

tolerance ie inhibition of disease establishment, immunity ie inhibition of transport or the presence of antiviral agents and the like. Alternatively, genes coding for virus resistance in plants can be actively switched on by way of inducing expression of a gene or genes encoding for a viral resistance. An example of such a system includes the hypersensitive response.

So-called hypersensitive responses (HSR) in plants have been reported and are generally characterized by death of plant cells in the vicinity of the penetrating pathogen shortly after infection. Movement of the pathogen through infected or invaded cells is restricted or blocked due to necrosis of the invaded cell and/or cells in the environs of the invaded cell(s). In addition, HSR involves a cascade of additional or secondary defense responses and the accumulation of certain proteins and secondary metabolites, leading to a general increased level of resistance to attack by pathogens. HSR reactions to invading organisms are generally thought to involve a resistance gene product in the plant cell which recognizes and interacts with an elicitor element, ie the product of an avirulence gene of a pathogen. Elicitor element recognition in the cells of a resistant plant triggers an HSR reaction which in its turn restricts the pathogen infection to a single cell or cells, or at most to a few plant cells in the immediate vicinity thereof.

An example of HSR-mediated resistance to virus infection is that of tobacco plants harbouring the N' resistance gene to tobamoviruses such as TMV and ToMV, which contain the coat protein avirulence gene. Thus far, more than twenty single dominant HSR-type resistance genes have been identified, and are present in many agronomically important crops including tobacco, tomato, potato, pepper, lettuce, and the like.

Despite the apparent abundance of resistance sources to certain viruses, many crops still lack effective resistance genes to

important viral pathogens [Fraser, R.S.S. (1992). *Euphytica* 63:175]. Searching of wild type germplasm collections has identified only a few suitable sources of viral resistance capable of being introduced successfully into agronomically important crops. An example is the absence of vertical resistance genes to cucumber mosaic virus (CMV) in many agronomically important crop types including but not limited to tomato, pepper, cucumber, melon, lettuce and the like.

Plant breeders continuously try to develop varieties of crop plant species tolerant to or resistant to specific virus strains. In the past, virus resistance conferring genes have been transferred from wild types related to commercial plants into commercial varieties through breeding. The transfer of an existing resistance in the wild from the wild type gene pool to a cultivar is a tedious process in which the resistance conferring gene(s) must first be identified in a source (donor) plant species and then combined into the gene pool of a commercial variety. Resistance or tolerance generated in this way is typically active only against one or at best a few strains of the virus in question. A further disadvantage is that the breeding programme generally takes a long time, measured in years, in getting to agronomically useful plants.

In an alternative, a system referred to as "cross-protection" has been employed. Cross-protection is a phenomenon in which infection of a plant with one strain of a virus protects that plant against superinfection with a second related virus strain. The cross-protection method preferentially involves the use of avirulent virus strains to infect plants, which act to inhibit a secondary infection with a virulent strain of the same virus. However, the use of a natural cross-protection system can have several disadvantages. The method is very labour intensive because it requires inoculation of each individual plant crop, and carries the risk that an avirulent strain may mutate to a virulent strain, thus becoming a causal agent for crop disease in itself. A further possible hazard is

that an avirulent virus strain in one plant species can act as a virulent strain in another plant species.

Genetically engineered cross-protection is a form of virus resistance which phenotypically resembles natural cross-protection, but is achieved through the expression of genetic information of a viral coat protein from the genome of a genetically manipulated plant. It is known that expression of the tobacco mosaic virus strain U1 (TMV-U1) coat protein gene from the genome of a transgenic plant can result in a delay of symptom development after infection with any TMV strain. Similarly, coat protein-mediated protection has also been obtained for alfalfa mosaic virus (AMV), potato virus X (PVX) and cucumber mosaic virus (CMV). For some plant viruses, eg luteoviruses, it is difficult to obtain detectable amounts of the corresponding coat protein in a transgenic plant, and consequently, virus resistance is generally lowered. Furthermore, any alleged degree of protection requires that the plant produces coat protein continually and thus imposes an energy burden on the plant. As a result of such limitations the commercial value of such technology remains unclear.

A further example of genetically engineered virus resistance includes the introduction of plant viral satellite RNA wherein expression of incorporated genetic material modifies the plant virus or its effects.

An object of the present invention is to provide an alternative more reliable engineered virus resistance strategy in plants to those engineered resistances known in the art, based on direct pathogen induced expression of molecules in target tissues of a plant before the invading pathogen can establish itself in the host plant.

Another object of the invention is to combine genetic engineering plant transformation technology with naturally existing plant viral defense mechanisms in plant tissue.

Detailed Description

According to the present invention there is provided a plant virus DNA construct capable of encoding directly or indirectly for a minus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by an invading virus such that at least one eliciting element is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus.

In another embodiment of the invention there is provided a plant virus recombinant DNA construct capable of encoding for a plus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by an invading virus producing as a result of such interaction, a plus sense RNA molecule which is capable of encoding for at least one eliciting element capable of eliciting a natural plant defense in the plant on invasion of the plant by the said invading virus.

The plant virus DNA construct can be derived from any virus source capable of attacking a plant, however it is preferred that the plant virus DNA is derived from any virus source which is known to attack, is suspected of attacking or is capable of attacking an agronomically attractive plant type. It can be a natural plant virus DNA suitably modified for expression or it may be derived synthetically. The plant virus DNA should be capable of encoding for transcription into an RNA sequence complementary (ie minus sense) to a viral RNA (ie plus sense) in plant cells. In addition, the plant virus DNA should contain a portion or segment thereof which when transcribed to yield minus sense RNA and further transcribed to plus sense RNA, upon translation of the plus sense RNA, is capable of giving rise to at least one eliciting element or part thereof sufficient to elicit a natural plant defense mechanism against an invading virus. Suitable plant virus DNA or RNA sources are those derived from plant viruses capable of invading plant

types such as tomatoes, peppers, melons, lettuces, cauliflowers, broccolis, cabbages, brussels sprouts, sugar beets, corn (maize), sweetcorn, onions, carrots, leeks, cucumbers, tobacco and the like. Also included as plant virus DNA or RNA sources are those derived from plant viruses capable of invading plant types from ornamental crops such as Impatiens, begonia, petunia, pelargoniums (geraniums, viola, cyclamen, verbenas, vinca, tagetes, primula, saintpaulia and the like.

A minus sense RNA molecule is one which contains at least a cistron or part thereof corresponding to at least a portion of the said plant virus DNA and is capable of giving rise to a plus sense RNA molecule transcribable from the said minus sense RNA molecule which is capable of coding for and giving rise to at least one eliciting element or part thereof in plant cells. The minus sense RNA may be directly transcribable from the said plant virus DNA or it may be transcribable from a plus sense RNA derived from the said DNA. As such, the minus sense RNA transcribable from a plus sense RNA is referred to, for the purposes of the present invention, as being indirectly transcribable from the said DNA. The orientation or polarity of the cistron or cistrons or parts thereof located on the minus sense RNA molecule can be such that the eliciting element may not be directly coded for after transcription from the plant virus DNA construct. The genetic code of the cistron or cistrons or parts thereof is located on the complementary strand to the minus sense RNA molecule i.e. the plus sense RNA. The cistron coding for an eliciting element becomes available for translation when the minus sense viral RNA sequence is replicated by an RNA dependent RNA polymerase encoded for by an invading virus to yield a plus sense RNA molecule.

Minus sense RNA herein also includes those RNA molecules which can be described as having ambisense characteristics, such as RNA molecules from tospoviruses and the like. In such cases, the minus sense RNA contains at least a cistron corresponding

to a portion of the said plant virus DNA and is capable of giving rise to a plus sense RNA transcribable from the said minus sense RNA which is capable of coding for and giving rise to at least one eliciting element or part thereof in plant cells.

A plus sense viral RNA molecule is one which is capable of directly or indirectly encoding at least one eliciting element or part thereof capable of being expressed in, and having a natural or engineered plant defense eliciting activity in plant cells. A plus sense RNA molecule is also one which is complementary to a viral minus sense RNA and is capable of giving rise directly or indirectly to at least one elicitor element upon translation in plant cells. Thus, a viral sense RNA molecule can be viewed as a complementary RNA molecule to a minus sense RNA molecule.

Plus sense RNA herein also includes those RNA molecules which can be described as having ambisense characteristics. In such cases, the plus sense RNA contains at least a cistron corresponding to a portion of the said plant virus DNA and is capable of directly coding for and giving rise to at least one eliciting element or part thereof in plant cells.

The amount of eliciting element which is expressed in the plant cell must be sufficient to elicit at least a cellular plant defense response against an invading virus resulting in a natural or engineered plant reaction effective in blocking or restricting further viral action. Thus, plus sense RNA molecules whether they be the complement of a minus sense RNA or an ambisense RNA must be capable of giving rise to elicitor elements which are capable of triggering or eliciting a natural or engineered plant defence response, whether that be through direct translation or through interaction with a viral RNA dependent RNA polymerase (eg via a generated subgenomic RNA). There can be one or more eliciting elements ultimately encoded by the plus sense RNA depending on the type of plant defense

response/plant defence responses being elicited. The viral plus sense RNA sequence is preferably one wherein at least a viral cistron has been replaced by at least a cistron coding for an eliciting element capable of being expressed in plant cells, and having a natural or engineered plant defense eliciting activity in plant tissue.

The eliciting element can be any element translatable from a plus sense RNA cistron derivable from a plant virus DNA as hereinbefore described, and can be a protein, polypeptide, or peptide or fragments thereof. Examples of preferred eliciting elements include the so-called elicitor proteins and/or cell inhibitory proteins.

An elicitor protein is one which if present in plant tissue, is capable of eliciting, triggering, or inducing a hypersensitive response (HSR), that is a natural plant defense mechanism against invading pathogens such as viruses. Elicitor proteins can be of plant virus origin, such as coat proteins, proteins involved in cell-to-cell movement, helicases, RNA-dependent RNA polymerases and the like. In addition, elicitor proteins can originate from or be derived from other plant pathogens such as bacteria, fungi, nematodes and the like.

A cell inhibitory protein is a protein which if present in plant tissue, has a detrimental effect on the plant cell, leading to inhibition of cell growth eg cell division, and/or cell death. Cell inhibitory agents include but are not restricted to ribonucleases, proteinases, ribosomal inhibitory proteins, cell wall degrading proteins and the like.

The minus and plus sense RNA molecules can be viewed as plant virus RNAs since they are derived from a plant DNA construct as hereinbefore described and comprise the genome or a segment of the genome of a plant virus. In such plant virus RNAs, selected nucleotide fragments can be replaced by others or can be deleted. Replacement and/or deletion of nucleotides or

segments comprised of nucleotides should be such so as not to interfere with the capability of the RNA molecule to multiply or replicate in virus-infected plant cells. Also, replacement and/or deletion of nucleotides, codons or segments comprised of nucleotides should be such so as not to interfere with the ability of the RNA dependent RNA polymerase of the invading virus to recognise and act upon an RNA molecule (in plus or minus sense orientation), and thereby initiating the sequence of events as described herein leading to the production of an effective amount of an eliciting element capable of eliciting a natural or engineered plant defense response. Examples of suitable plant virus RNA molecules include, but are not limited to genomic RNA molecules or segments thereof selected from the group comprising potyviruses, potexviruses, tobamoviruses, luteoviruses or genomic RNA or segments thereof of cucumoviruses, bromoviruses, tospoviruses and the like.

The plant virus DNA is under expression control of a promoter capable of functioning in plants and includes a terminator capable of functioning in plants.

A promoter is the nucleotide sequence upstream from the transcriptional initiation site and which contains all the regulatory regions required for transcription. Examples of promoters suitable for use in DNA constructs of the present invention include viral, fungal, bacterial, animal and plant-derived promoters capable of functioning in plant cells. A preferred promoter should express the DNA constitutively, that is in all living tissues of the plant. It will be appreciated that the promoter employed should give rise to the expression of the viral plant DNA at a rate sufficient to produce the amount of RNA capable of encoding for at least an elicitor element capable of eliciting a natural plant defense in a transformed plant on invasion of the plant by a virus. The required amount of RNA to be transcribed may vary with the type of plant. Examples of suitable promoters include the cauliflower mosaic virus 35S (CaMV 35S) and 19S (CaMV 19S)

promoters, the nopaline synthase and octopine synthase promoters, the heat shock 80 (hsp80) promoter and the like.

A terminator is contemplated as (A) a DNA sequence downstream of the viral DNA, coding for transcription into an RNA sequence which is capable of autocatalytical, self cleavage, to release the terminator sequences from the recombinant viral RNA sequence, followed by (B) a DNA sequence at the end of a transcriptional unit which signals termination of transcription. These elements are 3'-non-translated sequences containing polyadenylation signals, which act to cause the addition of poly adenylate sequences to the 3' end of primary transcripts. Examples of sequences mentioned under (A) include self-cleaving RNA molecules or ribozymes such as ribonuclease P, *Tetrahymena* L-19 intervening sequence, hammerhead ribozymes, *Hepatitis delta virus* RNA, *Neurospora* mitochondrial VS RNA and the like [Symons, R.H. (1992). *Ann. Rev. Biochem.* 61:641]. Sequences mentioned under (B) may be isolated from fungi, bacteria, animals and/or plants. Examples, particularly suitable for use in the DNA constructs of the invention include the nopaline synthase polyadenylation signal of *Agrobacterium tumefaciens*, the 35S polyadenylation signal of CaMV and the zein polyadenylation signal from *Zea mays*.

A DNA or RNA sequence is complementary to another DNA or RNA sequence if it is able to form a hydrogen-bonded complex with it, according to rules of base pairing under appropriate hybridization conditions. For the purposes of the present invention appropriate hybridization conditions may include but are not limited to, for example, an incubation for about 16 hours at 42°C, in a buffer system comprising 5 x standard saline citrate (SSC), 0.5% sodium dodecylsulphate (SDS), 5 x Denhardt's solution, 50% formamide and 100 µg/ml carrier DNA or RNA (hereinafter the buffer system), followed by washing 3x in buffer comprising 1 x SSC and 0.1% SDS at 65°C for approximately an hour each time. Thus the hybridisation signal obtained for an RNA or DNA molecule, for example an

autoradiogram reading, should be sufficiently clear to the man skilled in the art so as to suggest that an RNA or DNA molecule obtained could usefully be employed in the construction of plant virus DNA constructs suitable for use in the invention. Naturally, such an RNA or DNA molecule should be capable of the requisite activity as described herein. Thus replacement and/or deletion of nucleotides, codons or segments comprised of nucleotides should be such so as not to interfere with the ability of a DNA construct of the invention to code for a minus sense RNA molecule as herein described which is capable of being recognised by and of interaction with an RNA dependent RNA polymerase of an invading virus and thereby initiating the sequence of events as described herein leading to the production of an effective amount of an eliciting element capable of eliciting a natural or engineered plant defense response.

Suitable hybridization conditions employed in the present invention can involve incubation in a buffer system for about 16 hours at 49°C and washing 3x in a buffer comprising 0.1 x SSC and 0.1% SDS at 55°C for about an hour each time. More preferably, hybridization conditions can involve incubation in a buffer system for about 16 hours at 55°C and washing 3x in a buffer comprising 0.1 x SSC and 0.1% SDS at 65°C for approximately an hour each time. Naturally, any RNA or DNA molecule subjected to such hybridisation conditions should be capable of the requisite activity as described herein.

The invention also provides a vector capable of introducing the DNA construct of the invention into plants and methods of producing such vectors. The term vector employed herein refers to a vehicle by means of which DNA molecules or fragments thereof can be incorporated into a host organism. Suitable vehicles include plasmids, naked DNA introduced using micro-injection, particle guns, and the like [Offringa (1991). PhD thesis, State University Leiden, The Netherlands, Ch1:pages 7-28].

The term plants as used herein is used in a wide sense and refers to differentiated plants as well as undifferentiated plant material such as protoplasts, plant cells, seeds, plantlets and the like which under appropriate conditions can develop into mature plants, the progeny thereof and parts thereof such as cuttings and fruits of such plants.

The invention further provides plants comprising in their genome a DNA construct of the invention, and methods of producing such plants.

The plants according to the invention have reduced susceptibility to diseases caused by the respective viruses and do not have the disadvantages and limitations of plants obtained by classical methods and genetic engineering methods as discussed herein.

The invention is illustrated by the following non-limiting examples and accompanying figures.

Figure 1: Schematic representation of the interaction of pathogen and plant encoded proteins leading to induction of an HSR response.

Figure 2: Schematic representation of CMV resistant tobacco or tomato plants, obtained by expression of a minus-sense CMV RNA 3 molecule in which the MP gene is replaced by a gene coding for an elicitor (ToMV CP or P30) or a cell inhibitory protein (RNase T1).

Figure 3: Schematic representation of CMV resistant tobacco or tomato plants obtained by expression of a plus-sense CMV RNA 3 molecule in which the CP gene is replaced by a gene coding for an elicitor (ToMV CP or P30) or a cell inhibitory protein (RNase T1).

Sequence ID 1: Chimaeric cucumber mosaic virus RNA 3.

Sequence ID 2: Coat protein of ToMV (corresponding to nucleotides from positions 123-600 of Seq. ID. No.1).

Sequence ID 3: Coat protein of cucumber mosaic virus corresponding to nucleotide positions from 897-1550 of Seq. ID. No 1.

Sequence ID 4: Chimaeric cucumber mosaic virus RNA 3.

Sequence ID 5: RNase T1 corresponding to positions 123-437 of Seq. Id No. 4.

Sequence ID 6: Chimaeric cucumber mosaic virus RNA 3, coding for P30 of ToMV.

Sequence ID 7: P30 of ToMV corresponding to nucleotide positions 123-914 of Seq. ID No.7.

Sequence ID 8: Chimaeric tomato spotted wilt virus S RNA, coding for the coat protein of ToMV and the non-structural protein, NSs in opposite polarity.

Sequence ID 9: The non-structural protein, NSs (in opposite polarity) corresponding to nucleotide positions 1141-2543 of Seq ID No.8.

Examples

All CMV, TSWV, and ToMV RNA-derived sequences presented here are depicted as DNA sequences for the sole purpose of uniformity. It will be appreciated that this is done for convenience only.

Cultivars of *Nicotiana tabacum* and *Lycopersicon esculentum*, used in plant transformation studies, are grown under standard greenhouse conditions. Axenic explant material is grown on standard MS

media [Murashige and Skoog (1962). *Physiol. Plant* 15:473] containing appropriate phytohormones and sucrose concentrations.

E. coli bacteria are grown on rotary shakers at 37°C in standard LB-medium. *Agrobacterium tumefaciens* strains are grown at 28°C in MinA medium supplemented with 0.1 % glucose [Ausubel et al., (1987). *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Inter-sciences, New York, Chichester, Brisbane, Toronto, and Singapore].

In all cloning procedures the *E. coli* strain JM83 , (F⁻, Δ(lac-pro), ara, rpsL, Ø80, dlacZM15) is used as the preferred recipient for recombinant plasmids.

Binary vectors are conjugated to *Agrobacterium tumefaciens* strain LBA 4404, a strain containing the Ti-plasmid vir region, [Hoekema et al (1983). *Nature* 303: 179] in standard triparental matings using the *E. coli* HB101, containing the plasmid pRK2013 as a helper strain. [Figurski and Helinski, (1979). *Proc. Natl. Acad. Sci. USA* 76: 1648]. Appropriate *Agrobacterium tumefaciens* recipients are selected on media containing rifampicin (50 µg/ml) and kanamycine (50 µg/ml).

Cloning of fragments in the vectors pUC19 [Yanish-Perron et al (1985). *Gene* 33: 103], pBluescript (Stratagene), pBIN19 [Bevan et al (1984). *Nucl. Acids Res.* 12: 8711] or derivatives, restriction enzyme analysis of DNA, transformation to *E. coli* recipient strains, isolation of plasmid DNA on small as well as large scale, nick-translation, *in vitro* transcription, DNA sequencing, Southern blotting and DNA gel electrophoresis are performed according to standard procedures [Maniatis et al (1982).

Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York; Ausubel et al supra, (1987)].

DNA amplification using the polymerase chain reaction (PCR) was performed as recommended by the supplier of the *Taq* polymerase (Perkin Elmer Cetus). Amplification of RNA by reverse transcription and subsequent standard DNA amplification was performed using the Gene Amp RNA PCR as recommended by the supplier (Perkin Elmer Cetus).

Example 1: Isolation of CMV particles and genetic material therein

A CMV serogroup I is isolated from squash and maintained on squash by mechanical passaging. Virus is purified from systemically infected squash leaves essentially following the procedure according to Francki et al [(1979) CMI/AAB Descr. of Plant Viruses 213]. Approximately 100 µg of virus in a volume of 250 µl is extracted with phenol, then with a mixture of phenol and chloroform and finally with chloroform. RNA is precipitated with ethanol and collected by centrifugation. The pellet is dissolved in 20 µl of water.

Example 2: Isolation of ToMV particles and genetic material therein

A ToMV isolate from tomato, is maintained on tobacco via mechanical passaging. Virus is purified from systemically infected tobacco leaves essentially following the method essentially according to Hollings & Huttinga [(1976) CMI/AAB Descr. of Plant Viruses 156]. Approximately 200 µg of virus in a volume of 300 µl is extracted with phenol, then with a mixture of phenol and chloroform and finally with chloroform. The RNA is precipitated with ethanol and collected by centrifugation. The pellet is dissolved in 50 µl of water.

Example 3: Molecular cloning of CMV RNA 3

The sequence of RNA 3 of CMV is isolated using RNA-based PCR on purified CMV RNA (Perkin Elmer Cetus supra). Two primers are designed, ZUP069:

(5' TTTGGATCCA CGTGGTCTCC TTTTGGAG 3'),

which is complementary to the first 16 nucleotides at the 3' end of RNA 3 of CMV (Seq. Id No.1), and ZUP068 :

(5' TTTGGATCCG TAATCTTACC ACT 3')

which is identical to the first 14 nucleotides at the 5' end of RNA 3 of CMV (Seq. Id. No.1). Both primers contain BamH1 restriction sites to enable further cloning of the amplified DNA molecules. Purified CMV RNA is subjected to the Gene Amp RNA PCR, and the resulting PCR fragment is isolated from an agarose gel and cloned into Sma1-linearized pUC19, yielding the recombinant plasmid pZU181.

Example 4: Molecular cloning of TSWV S RNA

A cDNA clone containing almost the complete TSWV S RNA-specific sequence was constructed by fusion of cDNA clones 520 and 614 on the unique EcoR1 site yielding pTSWV-S1 [De Haan et al (1990). J. Gen. Virol. 71: 1001]. The complete sequence of TSWV S RNA is isolated using RNA-based PCR on purified pTSWV-S1 DNA (Perkin Elmer Cetus supra). Two primers are designed, ZUP250:

5' (TTTGGATCCA GAGCAATCGT GTCAATTTTG TGTTCATACC TTAAC) 3'

which comprises 36 nucleotides identical to the first 36 nucleotides at the 5' end of TSWV S RNA (Seq. Id. No.8), and ZUP251:

5' (TTTGGATCCA GAGCAATTGT GTCAGAATTT TGTTCATAAT CAAACCTCAC TT) 3'

which comprises 43 nucleotides complimentary to the first 43 nucleotides at the 3' end of TSWV S RNA (Seq. Id. No.8). Both primers contain BamH1 restriction sites to enable further cloning of the amplified DNA molecules. The resulting PCR fragment is isolated from an agarose gel and cloned into Sma1-linearized pUC19, yielding the recombinant plasmid pTSWV-S2.

Example 5: Molecular cloning of the CP and P30 genes of ToMV

The sequence of the genes corresponding to the coat protein (CP) and P30 of ToMV is isolated using RNA-based PCR. Primer ZUP112 spans either side of the translational start codon of the CP gene of ToMV RNA:

5' GTATTAACCA TGGCTTACTC 3' (comprising 13 nucleotides identical to nucleotides 121-133 of Seq. Id. No.1) and

primer ZUP113 spans either side of the translational stop codon of the CP gene of ToMV RNA:

5' GCACCCATGG ATTTAAGATG 3' (comprising 16 nucleotides complementary to nucleotides 595-610 of Seq. Id. No.1), and

primer ZUP117 spans either side of the translational start codon of the P30 gene of ToMV RNA:

5' TATTTCTCCA TGGCTCTAGT 3' (comprising 13 nucleotides identical to nucleotides 121-133 of Seq. Id. No.6,) and

primer ZUP118 spans either side of the translational stop codon of the P30 gene of ToMV RNA:

5' GAGTAAGCCA TGGTTAATAC 3' (comprising 13 nucleotides complementary to nucleotides 911-923 of Seq. Id. No.6)

The primers contain NcoI restriction sites to enable further cloning of the amplified DNA molecules. Purified ToMV RNA is subjected to the Gene Amp RNA PCR. Resulting PCR fragments are isolated from an agarose gel and cloned into SmaI-linearized pUC19, yielding the recombinant plasmids pZU183 (containing the CP gene) and pZU206 (containing the P30 gene).

Example 6: Synthesis of the ribonuclease T1 gene

The sequence of the gene corresponding with ribonuclease T1 is synthesized on a commercial DNA synthesizer (Pharmacia LKB, Gene assembler plus) as primer ZUP110 (comprising nucleotides identical to nucleotides 121-293 of Seq. Id No.4):

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5' TTTCCATGGC ATGCGACTAC ACTTGCGGTT CTAACGCTA CTCTTCTTCA
   GACGTTTCTA CTGCTCAAGC TGCCGGATAT AACTTCACG AAGACGGTGA
   AACTGTTGGA TCTAATTCTT ACCCACACAA ATACAACAAC TACGAAGGTT
   TTGATTTCTC TGTGAGCTCT CCCTAC 3'
```

and primer ZUP111 (comprising nucleotides complementary to nucleotides 278-446 of Seq. Id. No.4):

```
5' GGGCCATGGT TATGTACATT CAACGAAGTT GTTACCAGAA GCACCA TTT
   GAGTGATAAC ACCAGCATGT TGGTTGTTTT CGTTGAAGAC GACAGGATTT
   GCACCTGGAG AAGGACCAGA GTAAACATCA CCGCTAGAGA GGATAGGCCA
   TTCGTAGTAG GGAGAGCTCA C 3'
```

Both primers contain NcoI restriction sites to enable further cloning of the amplified DNA molecules. The primers are annealed and subjected to a standard DNA PCR. The amplified DNA fragment is isolated from an agarose gel and cloned into SmaI-linearized pUC19, yielding the recombinant plasmid pZU230.

Example 7: Construction of an expression vector pZU-A

The 35S cauliflower mosaic virus (CaMV) promoter fragment is isolated from the recombinant plasmid pZ027, a derivative of pUC19 carrying as a 444 bp HindIII-PstI fragment the HincII-HphI region of the 35S promoter of CaMV strain Cabb-S [Franck et al (1980). Cell 21: 285-294]. The nucleotide sequences of CaMV strains are very similar for the different strains. The 35S promoter fragment is excised from pZ027 as a 472 bp EcoRI-PstI fragment which contains: a part of the polylinker region,

437 bp of the non-transcribed region and the transcription initiation site and 7 bp of the non-translated leader region but not containing any 35S translational initiators. The 35S promoter fragment is ligated using T4 ligase into EcoRI-PstI linearized pZ0008. The plasmid pZ0008 carries the nopaline synthase (NOS) polyadenylation signal as a 270 bp PstI-HindIII fragment. The resulting recombinant plasmid pZU-A carries the 35S promoter, a unique PstI site and the NOS terminator [Gielen et al (1991) Bio/Technology 10:1363].

Example 8: Construction of a plant transformation vector, which yields a transcript which replicates upon infection with CMV.

The 5' end of the minus-sense RNA 3 of CMV is fused directly to the transcription initiation site of the CaMV 35S promoter using two primers ZUP148:

5' CCACGTCTTC AAAGCAAG 3' (complementary to nucleotides of the CaMV 35S promoter), and primer ZUP146:

5'CTTCGCACCT TCGTGGGGGC TCCAAAAGGA GACCACCTCT CCAAATGAAA 3'
(comprising nucleotides complementary to nucleotides 1860-1827 of Seq. Id. No.1)

with pZU-A as a template in a standard DNA PCR reaction. The amplified DNA fragment is digested with EcoRV and cloned in EcoRV linearized pZU-A. The resulting plasmid is digested with BstX1 and Pst1 and purified on an agarose gel. pZU181 is digested with Pst1 and BstX1, the 2.1 kb insert DNA is purified on an agarose gel and subsequently cloned into the gel-purified pZU-A derivative, yielding pCMV3AS-1.

The movement protein (MP) coding domain of pCMV3AS-1 is replaced by a unique NcoI cloning site and the axehead structure of the Hepatitis delta viral RNA is cloned downstream of the 3' end of the minus-sense RNA 3 of CMV, by PCR amplification of two DNA fragments using pCMV3AS-1 as a

template. The first DNA fragment is amplified using primers ZUP050:

5' AGCTGCTAAC GTCTTATTAA G 3' (comprising nucleotides complementary to nucleotides 1020-1039 of Seq. Id. No.1)

and ZUP329:

5' GTCTTTAGCA CCATGGTG 3' (comprising nucleotides identical to nucleotides 604-612 of Seq Id. No.1)

The DNA fragment is digested with *Nru*I and *Nco*I and a 411 bp long DNA fragment (position 607-1016 Seq. Id. No.1) is isolated from an agarose gel. The second DNA fragment is amplified using primers ZUP327:

5' GGAGAGCCAT GGCTCGGG 3' (comprising nucleotides complementary to nucleotides 115-126 of Seq. Id. No.1)

and ZUP350, a primer synthesised with nucleotides comprising nucleotides complementary to antigenomic hepatitis delta virus RNA as described by Perrotta AT & Been MD (1991) Nature Vol 350(4) pp434-436 ligated to nucleotides identical to nucleotides 1-14 (3' end of the primer) of Seq. Id. No. 1:

5'TTTCTGCAGA TCTTAGCCAT CCGAGTGGG CGTGCGTCCT CCTTCGGATG
CCCAGGTCGG ACCGCGAGGA GGTGGAGATG CCATGCCGAC CCGTAATCTT
ACCACT)3'.

The DNA fragment is digested with *Pst*I and *Nco*I and a 208 bp. long DNA fragment is isolated from an agarose gel. Both isolated DNA fragments are cloned in pCMV3AS-1, linearized with *Pst*I and *Nru*I, to yield pCMV3AS-2. Genes coding for elicitors (example 5) or cell inhibitory proteins (example 6) can be cloned as *Nco*I DNA fragments into the unique *Nco*I site of pCMV3AS-2. The resulting pCMV3AS-2 derived plasmids are digested with *Hind*III and the DNA fragments containing the

cn1maeric genes are isolated from an agarose gel and ligated into HindIII linearized pBIN19, resulting in binary plant transformation vectors pBINCMV3-CP, pBINCMV3-P30 and pBINCMV3-T1 respectively.

Example 9: Construction of a plant transformation vector, which yields a transcript which replicates upon infection with TSWV.

The 5' end of the minus-sense TSWV S RNA is fused directly to the transcription initiation site of the CaMV 35S promoter using two primers ZUP148 (Example 8), and primer ZUP255:

5' ACACAATTGC TCTCCTCTCC AAATGAAA 3' (comprising nucleotides identical to nucleotides 2608-2621 of Seq. Id. No.8)

with pZU-A as a template in a standard DNA PCR reaction. The amplified DNA fragment is digested with EcoRV and cloned in EcoR5 linearized pZU-A. The resulting plasmid is digested with MunI and PstI and purified on an agarose gel. pTSWV-S2 is digested with PstI and MunI, the 2.9 kb insert DNA is purified on an agarose gel and subsequently cloned into the gel-purified pZU-A derivative, yielding pTSWVSAS-1.

The N coding domain of pTSWVSAS-1 is replaced by a unique NcoI cloning site and the axehead structure of the Hepatitis delta viral RNA is cloned downstream of the 3' end of the minus-sense TSWV S RNA, by PCR amplification of two DNA fragments using pTSWVSAS-1 as a template. The first DNA fragment is amplified using primers ZUP252:

5' GACCCGAAAG GGACCAATTT C 3' (comprising nucleotides complimentary to nucleotides 911-930 of Seq Id. No.8)

and ZUP253:

5' TTTCCATGGC TGTAAGTTAA ATT 3' (comprising nucleotides identical to nucleotides 636-655 of Seq Id. No.8)

The DNA fragment is digested with *BalI* and *NcoI* and a 269 bp long DNA fragment (position 636-911 Sequence Id No.8) is isolated from an agarose gel. The second DNA fragment is amplified using primers ZUP254:

5' TTTCATGGT GATCGTAAAA G 3' (comprising nucleotides complementary to nucleotides 140-157 of Seq. Id No.8)

and ZUP255 a primer synthesised with nucleotides comprising nucleotides complementary to antigenomic hepatitis delta virus RNA as described by Perrotta AT & Been MD (1991) Nature Vol 350(4) pp434-436, ligated to nucleotides identical to nucleotides 1-14 (3' end of the primer) of Seq. Id. No. 8:

5' TTTCTGCAGA TCTTAGCCAT CCGAGTGGAC GTGCGTCCTC CTCGGATGC
CCAGGTCGGA CCGCGAGGAG GTGGAGATGC CATGCCGACC CAGAGCAATC
GTGTC 3'

The DNA fragment is digested with *PstI* and *NcoI* and a 245 bp. long DNA fragment is isolated from an agarose gel. Both isolated DNA fragments are cloned in pTSWVSAS-1, linearized with *PstI* and *BalI*, to yield pTSWVSAS-2. Genes coding for elicitors (example 5) or cell inhibitory proteins (example 6) are cloned as *NcoI* DNA fragments into the unique *NcoI* site of pTSWVSAS-2. The resulting pTSWVSAS-2 derived plasmids are digested with *XbaI* and the DNA fragments containing the chimaeric genes are isolated from an agarose gel and ligated into *XbaI* linearized pBIN19, resulting in binary plant transformation vectors pBINTSWVS-CP (Seq Id No.8), pBINTSWVS-P30 and pBINTSWVS-T1 respectively.

Example 10: Selection of suitable host plants

1) Tobacco, *Nicotiana tabacum* var. Samsun EN. A tobacco cultivar harboring the N' gene of *N. sylvestris* showing an HS response upon infection with ToMV. The CP of ToMV elicits a strong HSR defense reaction in this host.

2) Tomato, *Lycopersicon esculentum* var. ATV847, parental line for commercial hybrids Yaiza and Gemma. A tomato line harboring the Tm-2² resistance gene to ToMV. It has been demonstrated that the P30 of ToMV elicits a HS response in this resistant genotype [Fraser (1986) CRC Crit. Rev. Plant Sci. 3: 257; Keen (1990). Ann. Rev. Genet. 24: 447].

Example 11: Transformation of binary vectors to tobacco and tomato plant material

Methods to transfer binary vectors to plant material are well established and known to a person skilled in the art. Variations in procedures exist due to for instance differences in used *Agrobacterium* strains, different sources of explant material, differences in regeneration systems depending on as well the cultivar as the plant species used.

The binary plant transformation vectors as described above are used in plant transformation experiments according to the following procedures. Binary vector constructs are transferred by tri-parental mating to an acceptor *Agrobacterium tumefaciens* strain, followed by southern analysis of the ex-conjugants for verification of proper transfer of the construct to the acceptor strain, inoculation and cocultivation of axenic explant material with the *Agrobacterium tumefaciens* strain of choice, selective killing of the *Agrobacterium tumefaciens* strain used with appropriate antibiotics, selection of transformed cells by growing on selective media containing kanamycine, transfer of tissue to shoot-inducing media, transfer of selected shoots to root inducing media, transfer of plantlets to soil, assaying for intactness of the construct by southern analyses of isolated total DNA from the transgenic plant, assaying for proper function of the inserted chimaeric gene by northern analysis and/or enzyme assays and western blot analysis of proteins [Ausubel et al supra, (1987)].

Example 12: Expression of chimaeric sequences in tobacco and tomato plant cells

RNA is extracted from leaves of regenerated plants using the following protocol. Grind 200 mg leaf material to a fine powder in liquid nitrogen. Add 800 µl RNA extraction buffer (100 mM Tris-HCl (pH 8,0), 500 mM NaCl, 2 mM EDTA, 200 mM β-Mercaptoethanol, 0,4% SDS) and extract the homogenate with phenol, collect the nucleic acids by alcohol precipitation. Re suspend the nucleic acids in 0,5 ml 10 mM Tris-HCl (pH 8,0), 1 mM EDTA, add LiCl to a final concentration of 2 M, leave on ice for maximally 4 hours and collect the RNA by centrifugation. Re suspend in 400 µl 10 mM Tris-HCl (pH 8,0), 1 mM EDTA and precipitate with alcohol, finally re-suspend in 50 µl 10 mM Tris-HCl (pH 8,0), 1 mM EDTA. RNAs are separated on glyoxal/agarose gels and blotted to Genescreen as described by van Grinsven et al [(1986). Theor. Appl. Gen. 73:94-101]. Recombinant viral RNA sequences are detected using DNA or RNA probes labeled with [³²P], [³⁵S] or by using non-radioactive labeling techniques. Based on northern analysis, it is determined to what extent the regenerated plants express the chimaeric recombinant viral genes.

Plants transformed with recombinant viral DNA sequences are also subjected to western blot analysis after inoculation with the respective virus. Proteins are extracted from leaves of transformed plants by grinding in sample buffer according to Laemmli [(1970). Nature 244: 29]. A 50 µg portion of protein is subjected to electrophoresis in a 12,5 % SDS-polyacrylamide gel essentially as described by Laemmli supra, (1970). Separated proteins are transferred to nitrocellulose electrophoretically as described by Towbin et al [(1979). Proc. Natl. Acad. Sci. USA 76: 4350]. Transferred proteins are reacted with antiserum raised against purified ToMV particles or against purified P30 protein, according to Towbin et al supra, (1979). Based on the results of the western analysis,

it is determined that transformed plants do express elicitor proteins after inoculation with the respective virus.

Example 13: Resistance of tobacco and tomato plants against CMV or TSWV infection.

Transformed plants are grown in the greenhouse under standard quarantine conditions in order to prevent any infections by pathogens. The transformants are self-pollinated and the seeds harvested. Progeny plants are analyzed for segregation of the inserted gene and subsequently infected with CMV or TSWV by mechanical inoculation. Tissue from plants systemically infected with CMV or TSWV is ground in 5 volumes of ice-cold inoculation buffer (10 mM phosphate buffer) and rubbed in the presence of carborundum powder on the first two fully extended leaves of approximately 5 weeks old seedlings. Inoculated plants are monitored for symptom development during 3 weeks after inoculation.

Plants containing CMV Related DNA Sequences or TSWV related DNA sequences show reduced susceptibility to CMV or TSWV infection compared with untransformed control plants which show severe systemic CMV or TSWV symptoms within 7 days after inoculation.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.



SEQUENCE LISTING

Sequence ID No.1

Sequence type: Nucleotide

Sequence length: 1860 nucleotides

Strandness: Single stranded

Molecule type: Chimaeric cucumber mosaic virus RNA 3 coding for the CP of ToMV.

GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT	60
GAGTCGTUCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG	120
CCATGGCTTA CTCAATCACT TCTCCATCGC AATTTGTGTT TTTGTCATCT GTATGGGCTG	180
ACCCTATAGA ATTGTTAAAC GTTTGTACAA ATTCGTTAGG TAACCAGTTT CAAACACAGC	240
AAGCAAGAAC TACTGTTCAA CAGCAGTTCA GCGAGGTGTG GAAACCTTTC CCTCAGAGCA	300
CCGTCAGATT TCCTGGCGAT GTTTATAAGG TGTACAGGTA CAATGCAGTT TTAGATCCTC	360
TAATTACTGC GTTGCTGGGG TCTTTTCGATA CTAGGAATAG AATAATCGAA GTAGAAAACC	420
AGCAGAATCC GACAACAGCT GAAACGTTAG ATGCTACCCG CAGGGTAGAC GACGCTACGG	480
TTGCAATTCG GTCTGCTATA AATAATTTAG TTAATGAACT AGTAAGAGGT ACTGGACTGT	540
ACAATCAAAA TACTTTTGAA AGTATGTCTG GGTGGTCTG GACCTCTGCA CCTGCATCTT	600
AAATCCATGG TGTATTAGTA TATAAGTATT GTGAGTCTGT ACATAATACT ATATCTATAG	660
TGTCCTGTGT GAGTTGATAC AGTAGACATC TGTGACGCGA TGCCGTGTTG AGAAGGGAAC	720
ACATCTGGTT TTAGTAAGCC TACATCACAG TTTTGAGGTT CAATTCCTCA TACTCCCTGT	780
TGAGTCCCTT ACITTCTCAT GGATGCTTCT CCGCGAGATT GCGTTATTGT CTACTGACTA	840
TATAGAGAGT GTGTGTGCTG TGTTTTCTCT TTTGTGTCGT AGAATTGAGT CGAGTCATGG	900

ACAAATCTGA ATCAACCAGT GCTGGTCGTA ACCGTCGACG TCGTCCGCGT CGTGGTTCCC 960
GCTCCGCCCC CTCCTCCGCG GATGCTAACT TTAGAGTCTT GTCGCAGCAG CTTTCGCGAC 1020
TTAATAAGAC GTTAGCAGCT GGTCGTCCAA CTATTAACCA CCCAACCTTT GTAGGGAGTG 1080
AACGCTGTAA ACCTGGGTAC ACGTTCACAT CTATTACCCT AAAGCCACCA AAAATAGACC 1140
GTGGGTCTTA TTACGGTAAA AGGTTGTTAT TACCTGATTC AGTCACGGAA TATGATAAGA 1200
AACTTGTTTC GCGCATTCAA ATTCGAGTTA ATCCTTTGCC GAAATTCGAT TCTACCGTGT 1260
GGGTGACAGT CCGTAAAGTT CCTGCCTCCT CGGACTTATC CGTTGCCGCC ATCTCTGCTA 1320
TGTTTGCGGA CGCCGCATTT GGAGTCCAAG CTAACAACAA ATTGTTGTAT GATCTTTCGG 1380
CGGGAGCCTC ACCGGTACTG GTTTATCAGT ACATGCGCGC TGATATAGGT GACATGAGAA 1440
AGTACGCCGT CCTCGTGTAT TCAAAAGACG ATGCGCTCGA GACGGACGAG CTAGTACTTC 1500
ATGTTGACAT CGAGCACCAA CGCATTCCCA CATCTAGAGT ACTCCCAGTC TGATTCCGTG 1560
TTCCCAGAAC CCTCCCTCCG ATTTCTGTGG CGGGAGCTGA GTTGGCAGTT CTGCTATAAA 1620
CTGTCTGAAG TCACTAAACG TTTTACGGTG AACGGGTTGT CCATCCAGCT TACGGCTAAA 1680
ATGGTCAGTC GTGGAGAAAT CCACGCCAGC AGATTTACAA ATCTCTGAGG CGCCTTTGAA 1740
ACCATCTCCT AGGTTTTTTC GGAAGGACTT CGGTCCGTGT ACCTCTAGCA CAACGTGCTA 1800
GTCTTAGGGT ACGGGTGCCC CTTGTCTTCG CACCTTCGTG GGGGCTCCAA AAGGAGACCA 1860

Sequence ID No.2

Sequence type: Amino acid

Sequence length: 159 amino acids

Strandness: Single stranded

Molecule type: Coat protein of ToMV (corresponding to nucleotides from positions 123-599 of Seq. ID. No.1).

Met Ala Tyr Ser Ile Thr Ser Pro Ser Gln Phe Val Phe Leu Ser	15
Ser Val Trp Ala Asp Pro Ile Glu Leu Leu Asn Val Cys Thr Asn	30
Ser Leu Gly Asn Gln Phe Gln Thr Gln Gln Ala Arg Thr Thr Val	45
Gln Gln Gln Phe Ser Glu Val Trp Lys Pro Phe Pro Gln Ser Thr	60
Val Arg Phe Pro Gly Asp Val Tyr Lys Val Tyr Arg Tyr Asn Ala	75
Val Leu Asp Pro Leu Ile Thr Ala Leu Leu Gly Ser Phe Asp Thr	90
Arg Asn Arg Ile Ile Glu Val Glu Asn Gln Gln Asn Pro Thr Thr	105
Ala Glu Thr Leu Asp Ala Thr Arg Arg Val Asp Asp Ala Thr Val	120
Ala Ile Arg Ser Ala Ile Asn Asn Leu Val Asn Glu Leu Val Arg	135
Gly Thr Gly Leu Tyr Asn Gln Asn Thr Phe Glu Ser Met Ser Gly	150
Leu Val Trp Thr Ser Ala Pro Ala Ser	159

Sequence ID No.3

Sequence type: Amino acid

Sequence length: 218 amino acids

Strandness: Single stranded

Molecule type: Coat protein of cucumber mosaic virus
corresponding to nucleotide positions from 897-1550 of Seq. ID.
No 1.

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Met Asp Lys Ser Glu Ser Thr Ser Ala Gly Arg Asn Arg Arg Arg   15
Arg Pro Arg Arg Gly Ser Arg Ser Ala Pro Ser Ser Ala Asp Ala   30
Asn Phe Arg Val Leu Ser Gln Gln Leu Ser Arg Leu Asn Lys Thr    45
Leu Ala Ala Gly Arg Pro Thr Ile Asn His Pro Thr Phe Val Gly    60
Ser Glu Arg Cys Lys Pro Gly Tyr Thr Phe Thr Ser Ile Thr Leu   75
Lys Pro Pro Lys Ile Asp Arg Gly Ser Tyr Tyr Gly Lys Arg Leu   90
Leu Leu Pro Asp Ser Val Thr Glu Tyr Asp Lys Lys Leu Val Ser  105
Arg Ile Gln Ile Arg Val Asn Pro Leu Pro Lys Phe Asp Ser Thr  120
Val Trp Val Thr Val Arg Lys Val Pro Ala Ser Ser Asp Leu Ser  135
Val Ala Ala Ile Ser Ala Met Phe Ala Asp Gly Ala Ser Pro Val  150
Leu Val Tyr Gln Tyr Ala Ala Phe Gly Val Gln Ala Asn Asn Lys  165
Leu Leu Tyr Asp Leu Ser Ala Met Arg Ala Asp Ile Gly Asp Met  180
Arg Lys Tyr Ala Val Leu Val Tyr Ser Lys Asp Asp Ala Leu Glu  195
Thr Asp Glu Leu Val Leu His Val Asp Ile Glu His Gln Arg Ile  210
Pro Thr Ser Arg Val Leu Pro Val                                218

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Sequence ID No.4

Sequence type: Nucleotide

Sequence length: 1696 nucleotides

Strandness: Single stranded

Molecule type: Chimaeric cucumber mosaic virus RNA 3 coding for
RNase T1.

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GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT 60
GAGTCGTGCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG 120
CCATGGCATG CGACTACACT TCGGGTTCTA ACTGCTACTC TTCTTCAGAC GTTTCTACT 180
CTCAAGCTGC CGGATATAAA CTTCACGAAG ACGGTGAAAC TGTGGATCT AATTCTTACC 240
CACACAAATA CAACAACTAC GAAGGTTTTG ATTTCTCTGT GAGCTCTCCC TACTACGAAT 300
GGCCTATCCT CTCTAGCGGT GATGTTTACT CTGGTGGTTC TCCAGGTGCT GACCGTGTGCG 360
TCTTCAACGA AAACAACCAA CTAGCTGGTG TTATCACTCA CACTGGTGCT TCTGGTAACA 420
ACTTCGTTGA ATGTACATAA CCATGGTGTA TTAGTATATA AGTATTGTGA GTCTGTACAT 480
AATACTATAT CTATAGTGTC CTGTGTGAGT TGATACAGTA GACATCTGTG ACGCGATGCC 540
GTGTTGAGAA GGGAACACAT CTGGTTTTAG TAAGCCTACA TCACAGTTTT GAGGTTCAAT 600
TCCTCATACT CCCTGTTGAG TCCCTTACTT TCTCATGGAT GCTTCTCCGC GAGATTGCGT 660
TATTGTCTAC TGACTATATA GAGAGTGTGT GTGCTGTGTT TTCTCTTTTG TGTGCTAGAA 720
TTGAGTCGAG TCATGGACAA ATCTGAATCA ACCAGTGCTG GTCGTAACCG TCGACGTCGT 780
CCGCGTCGTG GTTCCCCTC CGCCCCCTCC TCCGCGGATG CTAACTTTAG AGTCTTGTCG 840
CAGCAGCTTT CGCGACTTAA TAAGACGTTA GCAGCTGGTC GTCCAACTAT TAACCACCCA 900
ACCTTTGTAG GGAGTGAACG CTGTAAACCT GGGTACACGT TCACATCTAT TACCCTAAAG 960
CCACCAAAAA TAGACCGTGG GTCTTATTAC GGTAAGAGGT TGTATTACC TGATTAGTC 1020
ACGGAATATG ATAAGAACT TGTTCGCGC ATTCAAATTC GAGTTAATCC TTTGCCGAAA 1080
TTCGATTCTA CCGTGTGGGT GACAGTCCGT AAAGTTCCTG CCTCCTCGGA CTTATCCGTT 1140
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GCCGCCATCT CTGCTATGTT TCGGACGGA GCCTCACCGG TACTGGTTTA TCAGTACGCC 1200
GCATTTGGAG TCCAAGCTAA CAACAAATTG TTGTATGATC TTTCGGCGAT GCGCGCTGAT 1260
ATAGGTGACA TGAGAAAGTA CGCCGTCCTC GTGTATTCAA AAGACGATGC GCTCGAGACG 1320
GACGAGCTAG TACTTCATGT TGACATCGAG CACCAACGCA TTCCACATC TAGAGTACTC 1380
CCAGTCTGAT TCCGTGTTCC CAGAACCCTC CCTCCGATTT CTGTGGCGGG AGCTGAGTTG 1440
GCAGTTCTGC TATAAACTGT CTGAAGTCAC TAAACGTTTT ACGGTGAACG GGTGTCCAT 1500
CCAGCTTACG GCTAAAATGG TCAGTCGTGG AGAAATCCAC GCCAGCAGAT TTACAAATCT 1560
CTGAGGCGCC TTTGAAACCA TCTCCTAGGT TTTTTCGGAA GGACTTCGGT CCGTGACCT 1620
CTAGCACAAC GTGCTAGTCT TAGGGTACGG GTGCCCTTG TCTTCGCACC TTCGTGGGGG 1680
CTCCAAAAGG AGACCA 1696

Sequence ID No. 5

Sequence type: Amino Acid

Sequence length: 105 amino acids

Strandness: Single stranded

Molecule type: RNase T1 corresponding to positions 123-437 of
Seq. Id No. 4.

Met Ala Cys Asp Tyr Thr Cys Gly Ser Asn Cys Tyr Ser Ser Ser	15
Asp Val Ser Thr Ala Gln Ala Ala Gly Tyr Lys Leu His Glu Asp	30
Gly Glu Thr Val Gly Ser Asn Ser Tyr Pro His Lys Tyr Asn Asn	45
Tyr Glu Gly Phe Asp Phe Ser Val Ser Ser Pro Tyr Tyr Glu Trp	60
Pro Ile Leu Ser Ser Gly Asp Val Tyr Ser Gly Gly Ser Pro Gly	75
Ala Asp Arg Val Val Phe Asn Glu Asn Asn Gln Leu Ala Gly Val	90
Ile Thr His Thr Gly Ala Ser Gly Asn Asn Phe Val Glu Cys Thr	105

Sequence ID No.6

Sequence type: Nucleotide

Sequence length: 2173 nucleotides

Strandness: Single stranded

Molecule type: Chimaeric cucumber mosaic virus RNA 3, coding for P30 of ToMV.

GTAATCTTAC CACTCGTGTG TGTGCGTGTG TGTGTGTCGA GTCGTGTTGT CCGCACATTT 60
GAGTCGTGCT GTCCGCACAT ATATTTTACC TTTTGTGTAC AGTGTGTTAG ATTTCCCGAG 120
CCATGGCTCT AGTTGTAAA GGTAAAGTAA ATATTAATGA GTTTATCGAT CTGTCAAAGT 180
CTGAGAACT TCTCCCGTCG ATGTTACGC CTGTAAAGAG TGTTATGGTT TCAAAGGTTG 240
ATAAGATTAT GGTCCATGAA AATGAATCAT TGTCTGAAGT AAATCTCTTA AAAGGTGTAA 300
AACTTATAGA AGGTGGGTAT GTTTGCTTAG TTGGTCTTGT TGTGTCCGGT GAGTGGGAATT 360
TCCCAGATAA TCGCCGTGGT GGTGTGAGTG TCTGCATGGT TGACAAGAGA ATGGAAAGAG 420
CGGACGAAGC CACTGCGG TCATATTACA CTGCTGCTGC TAAAAGCGG TTTCAGTTTA 480
AAGTGGTCCC AAATTACGGT ATTACAACAA AGGATGCAGA AAAGAACATA TGGCAGGTCT 540
TAGTAAATAT TAAAAATGTA AAAATGAGTG CGGGCTACTG CCCTTTGTCA TTAGAATTTG 600
TGTCTGTGTG TATTGTTTAT AAAAATAATA TAAAATTGGG TTTGAGGGAG AAAGTAACGA 660
GTGTGAACGA TGGAGGACCC ATGGAACTTT CGGAAGAAGT TGTGATGAG TTCATGGAGA 720
ATGTTCCAAT GTCGGTTAGA CTCGCAAAGT TTCGAACCAA ATCCTCAAAA AGAGGTCCGA 780
AAAATAATAA TAATTTAGGT AAGGGGCGTT CAGGCGGAAG GCCTAAACCA AAAAGTTTTG 840
ATGAAGTTGA AAAAGAGTTT GATAATTTGA TTGAAGATGA AGCCGAGACG TCGGTCGCGG 900
ATTCTGATTC GTATTAACCA TGGTGTATTA GTATATAAGT ATTGTGAGTC TGTACATAAT 960
ACTATATCTA TAGTGTCTG TGTGAGTTGA TACAGTAGAC ATCTGTGACG CGATGCCGTG 1020

TTGAGAAGGG AACACATCTG GTTTTAGTAA GCCTACATCA CAGTTTGTAG GTTCAATTCC 1080
TCATACTCCC TGTGAGTCC CTTACTTTCT CATGGATGCT TCTCCGCGAG ATTGCGTTAT 1140
TGTCTACTGA CTATATAGAG AGTGTGTGTG CTGTGTTTTT TCTTTTGTGT CGTAGAATTG 1200
AGTCGAGTCA TGGACAAATC TGAATCAACC AGTGCTGGTC GTAACCGTCG ACGTCGTCCG 1260
CGTCGTGGTT CCCGCTCCGC CCCCTCCTCC GCGGATGCTA ACTTTAGAGT CTTGTGCGAG 1320
CAGCTTTCGC GACTTAATAA GACGTTAGCA GCTGGTCGTC CAACTATTAA CCACCAACC 1380
TTTGTAGGGA GTGAACGCTG TAAACCTGGG TACACGTTCA CATCTATTAC CCTAAAGCCA 1440
CCAAAAATAG ACCGTGGGTC TTATTACGGT AAAAGGTTGT TATTACCTGA TTCAGTCACG 1500
GAATATGATA AGAACTTGT TTCGCGCATT CAAATTCGAG TTAATCCTTT GCCGAAATTC 1560
GATTCTACCG TGTGGGTGAC AGTCCGTAAA GTTCCTGCCT CCTCGGACTT ATCCGTTGCC 1620
GCCATCTCTG CTATGTTTGC GGACGCCGCA TTTGGAGTCC AAGCTAACAA CAAATTGTTG 1680
TATGATCTTT CGGCGGGAGC CTCACCGGTA CTGGTTTATC AGTACATGCG CGCTGATATA 1740
GGTGACATGA GAAAGTACGC CGTCCTCGTG TATTCAAAG ACGATGCGCT CGAGACGGAC 1800
GAGCTAGTAC TTCATGTTGA CATCGAGCAC CAACGCATTC CCACATCTAG AGTACTCCCA 1860
GTCTGATTCC GTGTCCCAG AACCCTCCCT CCGATTTCTG TGGCGGGAGC TGAGTTGGCA 1920
GTTCTGCTAT AACTGTCTG AAGTCACTAA ACGTTTTACG GTGAACGGGT TGTCATCCA 1980
GCTTACGGCT AAAATGGTCA GTCGTGGAGA AATCCACGCC AGCAGATTTA CAAATCTCTG 2040
AGGCGCCTTT GAAACCATCT CCTAGGTTTT TTCGGAAGGA CTCGGTCCG TGTACCTCTA 2100
GCACAACGTG CTAGTCTTAG GGTACGGGTG CCCCTTGTCT TCGCACCTTC GTGGGGGCTC 2160
CAAAAGGAGA CCA 2173

Sequence ID No.7

Sequence type: Amino acid

Sequence length: 264 amino acids

Strandness: Single stranded

Molecule type: P30 of ToMV corresponding to nucleotide positions 123-914 of Seq. ID No.6.

Met Ala Leu Val Val Lys Gly Lys Val Asn Ile Asn Glu Phe Ile	15
Asp Leu Ser Lys Ser Glu Lys Leu Leu Pro Ser Met Phe Thr Pro	30
Val Lys Ser Val Met Val Ser Lys Val Asp Lys Ile Met Val His	45
Glu Asn Glu Ser Leu Ser Glu Val Asn Leu Leu Lys Gly Val Lys	60
Leu Ile Glu Gly Gly Tyr Val Cys Leu Val Gly Leu Val Val Ser	75
Gly Glu Trp Asn Phe Pro Asp Asn Arg Arg Gly Gly Val Ser Val	90
Cys Met Val Asp Lys Arg Met Glu Arg Ala Asp Glu Ala Thr Leu	105
Gly Ser Tyr Tyr Thr Ala Ala Ala Lys Lys Arg Phe Gln Phe Lys	120
Val Val Pro Asn Tyr Gly Ile Thr Thr Lys Asp Ala Glu Lys Asn	135
Ile Trp Glu Val Leu Val Asn Ile Lys Asn Val Lys Met Ser Ala	150
Gly Tyr Cys Pro Leu Ser Leu Glu Phe Val Ser Val Cys Ile Val	165
Tyr Lys Asn Asn Ile Lys Leu Gly Leu Arg Glu Lys Val Thr Ser	180
Val Asn Asp Gly Gly Pro Met Glu Leu Ser Glu Glu Val Val Asp	195
Glu Phe Met Glu Asn Val Pro Met Ser Val Arg Leu Ala Lys Phe	210
Arg Thr Lys Ser Ser Lys Arg Gly Pro Lys Asn Asn Asn Asn Leu	225
Gly Lys Gly Arg Ser Gly Gly Arg Pro Lys Pro Lys Ser Phe Asp	240
Glu Val Glu Lys Glu Phe Asp Asn Leu Ile Glu Asp Glu Ala Glu	255
Thr Ser Val Ala Asp Ser Asp Ser Tyr	264

Sequence ID No.8

Sequence type: Nucleotide

Sequence length: 2621 nucleotides

Strandness: Single stranded

Molecule type: Chimaeric tomato spotted wilt virus S RNA, coding for the coat protein of ToMV and the non-structural protein, NSs in opposite polarity.

```
AGAGCAATCG TGTCAATTTT GTGTTTCATAC CTTAACTCTC AGTCTTACAA ATCATCACAT 60
TAAGAACCTA AGAAACGACT GCGGGATACA GAGTTGCACT TTCGCACCTT GAGTTACATA 120
CGGTCAAAGC ATATAACAAC TTTTACGATC ACCATGGCTT ACTCAATCAC TTCTCCATCG 180
CAATTTGTGT TTTTGTTCATC TGTATGGGCT GACCCTATAG AATTGTTAAA CGTTTGTACA 240
AATTCGTTAG GTAACCAAGT TCAAACACAG CAAGCAAGAA CTAAGTTTCA ACAGCAGTTC 300
AGCGAGGTGT GGAAACCTTT CCCTCAGAGC ACCGTCAGAT TTCCTGGCGA TGTTTATAAG 360
GTGTACAGGT ACAATGCAGT TTTAGATCCT CTAATTACTG CGTTGCTGGG GTCTTTTCGAT 420
ACTAGGAATA GAATAATCGA AGTAGAAAAC CAGCAGAATC CGACAACAGC TGAAACGTTA 480
GATGCTACCC GCAGGGTAGA CGACGCTACG GTTGCAATTC GGTCTGCTAT AAATAATTTA 540
GTTAATGAAC TAGTAAGAGG TACTGGACTG TACAATCAAA ATACTTTTGA AAGTATGTCT 600
GGGTTGGTCT GGACCTCTGC ACCTGCATCT TAAATCCATG GCTGTAAGTT AAATTATAAA 660
AAAGCCTATA AATATATAAA GCTTTCTTTA TCTTTATTGC TTGTGCTTGC TTAGTGTGTT 720
AAATTTTAAA TAAGTGTGTT TAATTAAAGT TTGCTTTCTG TGTGTGTGTC TTAATAAATA 780
ATAAAATAAC AAAAACAACG AAAACAAAAA ATAAATAAAA TAAAAATAAA ATAAAAATAA 840
AATAAAATAA AAATAAATAA AAAAATAAAA AACAAAAAAC AAAAAACAAA AACAAAACCC 900
```

AAATTTGGCC AAATTGGTCC CTTTCGGGTC TTTTGGTTT TTCGTTTTT AATTTTTTGT 960
TGTTTTTATT TCATTTTTTG ATTTTATTTT ATTTTAATTT TATTTTCATT TTTATTTTTT 1020
GTTTTTATGG TTTCTACTAG ACAGGAGGAA TTTGAAAGAG ATGACAAACA GAGAAATAAT 1080
TATAAGTAAA GAAAGAAAAT AAACATAACA TAATTAGAAA AAGCTGGACA AAGCAAGATT 1140
ATTTTGATCC TGAAGCATAC GCTTCCTTAA CCTTAGATTC TTTCTTTTTG ATCCCGCTTA 1200
AATCAAGCTT TAACAAAGAT TTTGCAACTG AAATAGATTG TGGAGAAATT TTAATTTCTC 1260
CTCTGGCAAA GTCTATCTTC CATGAAGGGA TTTGGATGCT GTCTAAGTAA GACATAGTTT 1320
GTGTGTTAGA TGGAAGACAT TCAAGTGTTT TTGAAAGGAA ATATTCCTT TTGTAGGCAT 1380
CTTCACTGTA ATTCAAGGTT CTTTCACCTA AATCTAACTT TCCAGGAGTT AGCTCAAGGT 1440
TGTTCAAAGT GTAGATGATT ACATCTTCTT GCAAGTTAGT TGCAAAGAAC TTGTGCAAAG 1500
ATGTGTGAGT TTCGAGCCAG AGCATTGGAA CCGATCCTTT GGGGTATGAA GGGTCATGAA 1560
CAATGTTGTA AGGCTCCTTT AAATCAGAAA ACATCATTGA TAATTCAAAA GGAGCTTTGC 1620
ATTTGCGAAT TGGGAGCTGA TGCTTGCAAA TAACAGTAAT GTTTAAAGCT GTCTCAACAC 1680
TGTTATGGTT TGGAATGCAG GCAATAGATA AATAAAATGT TTTGTTTGTT TCATCTCCTG 1740
CAACCTTGAA CAATTTCTGA ATGGAAACCT GCTTCAAAAC CTTTGAACC CTTAGCCAGA 1800
GGCTCAGCTT GAAATGAGAA TCAGTGAAG CTTGAGAGTT AGGCATGATG TTGTTTTCTG 1860
CTGACATGAG CAGAGATTTC ACTGCAAGAG AATTACAGT TCTGTTGTTG CTTTCAACTT 1920
GATTGAAATT TGGCTTGAAA CTGTACAGCC ATTCATGGAC ATTTCTGTTA GGAGATAGAA 1980
CATTCACTTT GCCTAAAGCC TGATTATAGC ACATCTCGAT CTTATAGGTA TGCTCTTTGA 2040
CACAAGACAA AGAGCCTTTG TTTGCAGCTT CAATGTATTT GTCATTGGGA ATTATGTCTT 2100
TTTCTTGGAG CTGGAATCGG TCTGTAATAT CAGATCTGTT CATGATAGAT TCAATAGAGT 2160
GGAGCTGGGC AGGAGACAAA ACCTTCAAAT GACCTTGATG TTTCACTCCG TTAGCATTGA 2220

CTGTATTGA GCAAACAGAT AGTGCCAGAA CAGAGTTATC AATATTGATG CTAAAATCAA 2280
TATCATCAAA AATAGGGATA TACACATGCT GAGAAAGAAA TCTCTTCTTC TTCACAGGGA 2340
AGATCCCTAC TTTGCAGTAT AGCCAAAGGA CTACTTTGCT TCTTGAATCA GAATACAGCT 2400
GGGTCTGAAC TAGTTGAGAA CCAGTACCAA GTTCATGAAT CCAGTAAGAA TCTACAACAG 2460
CTTTACCAGA TGCAGTTGAT CCCCAGACTG AAGCTCTTGT CTGAATGATC GACTCATAAA 2520
CACTTGAAGA CATTATGGTT ATTGGTACTG TGTCTTATT ACAGTATTGT GATTTTCTAA 2580
GTGAGGTTTG ATTATGAACA AAATTCTGAC ACAATTGCTC T 2621

Sequence ID No.9

Sequence type: Amino acid

Sequence length: 464 amino acids

Strandness: Single stranded

Molecule type: The non-structural Protein, NSs corresponding to nucleotide positions 1142-2533 (in opposite polarity) of Seq ID No.8.

Met Ser Ser Ser Val Tyr Glu Ser Ile Ile Gln Thr Arg Ala Ser	15
Val Trp Gly Ser Thr Ala Ser Gly Lys Ala Val Val Asp Ser Tyr	30
Trp Ile His Glu Leu Gly Thr Gly Ser Gln Leu Val Gln Thr Gln	45
Leu Tyr Ser Asp Ser Arg Ser Lys Val Val Leu Trp Leu Tyr Cys	60
Lys Val Gly Ile Phe Pro Val Lys Lys Lys Arg Phe Leu Ser Gln	75
His Val Tyr Ile Pro Ile Phe Asp Asp Ile Asp Phe Ser Ile Asn	90
Ile Asp Asn Ser Val Leu Ala Leu Ser Val Cys Ser Asn Thr Val	105
Asn Ala Asn Gly Val Lys His Gln Gly His Leu Lys Val Leu Ser	120
Pro Ala Gln Leu His Ser Ile Glu Ser Ile Met Asn Arg Ser Asp	135
Ile Thr Asp Arg Phe Gln Leu Gln Glu Lys Asp Ile Ile Pro Asn	150
Asp Lys Tyr Ile Glu Ala Ala Asn Lys Gly Ser Leu Ser Cys Val	165
Lys Glu His Thr Tyr Lys Ile Glu Met Cys Tyr Asn Gln Ala Leu	180
Gly Lys Val Asn Val Leu Ser Pro Asn Arg Asn Val His Glu Trp	195
Leu Tyr Ser Phe Lys Pro Asn Phe Asn Gln Val Glu Ser Asn Asn	210
Arg Thr Val Asn Ser Leu Ala Val Lys Ser Leu Leu Met Ser Ala	225
Glu Asn Asn Ile Met Pro Asn Ser Gln Ala Ser Thr Asp Ser His	240

Phe Lys Leu Ser Leu Trp Leu Arg Val Pro Lys Val Leu Lys Gln 255
Val Ser Ile Gln Lys Leu Phe Lys Val Ala Gly Asp Glu Thr Asn 270
Lys Thr Phe Tyr Leu Ser Ile Ala Cys Ile Pro Asn His Asn Ser 285
Val Glu Thr Ala Leu Asn Ile Thr Val Ile Cys Lys His Gln Leu 300
Pro Ile Arg Lys Cys Lys Ala Pro Phe Glu Leu Ser Met Met Phe 315
Ser Asp Leu Lys Glu Pro Tyr Asn Ile Val His Asp Pro Ser Tyr 330
Pro Lys Gly Ser Val Pro Met Leu Trp Leu Glu Thr His Thr Ser 345
Leu His Lys Phe Phe Ala Thr Asn Leu Gln Glu Asp Val Ile Ile 360
Tyr Thr Leu Asn Asn Leu Glu Leu Thr Pro Gly Lys Leu Asp Leu 375
Gly Glu Arg Thr Leu Asn Tyr Ser Glu Asp Ala Tyr Lys Arg Lys 390
Tyr Phe Leu Ser Lys Thr Leu Glu Cys Leu Pro Ser Asn Thr Gln 405
Thr Met Ser Tyr Leu Asp Ser Ile Gln Ile Pro Ser Trp Lys Ile 420
Asp Phe Ala Arg Gly Glu Ile Lys Ile Ser Pro Gln Ser Ile Ser 435
Val Ala Lys Ser Leu Leu Lys Leu Asp Leu Ser Gly Ile Lys Lys 450
Lys Glu Ser Lys Val Lys Glu Ala Tyr Ala Ser Gly Ser Lys 464

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A DNA construct capable of encoding directly or indirectly for a minus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant such that at least one protein, polypeptide or peptide eliciting a natural plant defence mechanism against invading pathogens is produced as a consequence of the interaction with the RNA dependent RNA polymerase encoded by the said invading virus, which construct is under expression control of a promoter and a terminator capable of functioning in plants.
2. A recombinant DNA construct capable of encoding for a plus sense RNA molecule capable of interacting with an RNA dependent RNA polymerase encoded for by a virus when invading a plant and replicating as a result of such interaction a plus sense RNA molecule which is capable of encoding for at least one protein, polypeptide or peptide eliciting a natural plant defence mechanism against invading pathogens, which construct is under expression control of a promoter and a terminator capable of functioning in plants.
3. A construct according to claim 1 comprising SEQ ID NO:1.
4. A construct according to claim 1 comprising SEQ ID NO:4.
5. A construct according to claim 1 comprising SEQ ID NO:6.
6. A construct according to claim 1 comprising SEQ ID NO:8.
7. A construct according to claim 1 or claim 2 wherein the eliciting element is capable of eliciting a hypersensitive response or the release of a cell inhibitory protein in a plant.



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8. A construct according to claim 1 or 2 wherein the defence mechanism is elicited by a protein is of plant virus, bacterial, fungal or nematode origin.
9. A construct according to claim 7 wherein the cell inhibitory protein is selected from the group comprising ribonucleases, proteinases, ribosomal inhibitory proteins, and cell wall degrading proteins.
10. A construct according to any one of claims 1 to 9 wherein the construct comprises a constitutive promoter selected from the group consisting of viral, fungal, bacterial and plant derived promoters.
11. A construct according to claim 10, wherein the promoter is selected from the group consisting of CaMV 19S, nopaline synthase, octopine synthase, heat shock 80 promoters.
12. Plants containing in their genome a construct according to any one of claims 1 to 11.
13. A process of preparing plants according to claim 12, which comprises:
 - A) Inserting into the genome of a plant cell a DNA construct according to claim 1;
 - B) obtaining transformed cells;
 - C) regenerating from the transformed cells genetically transformed plants.



Figure 1.

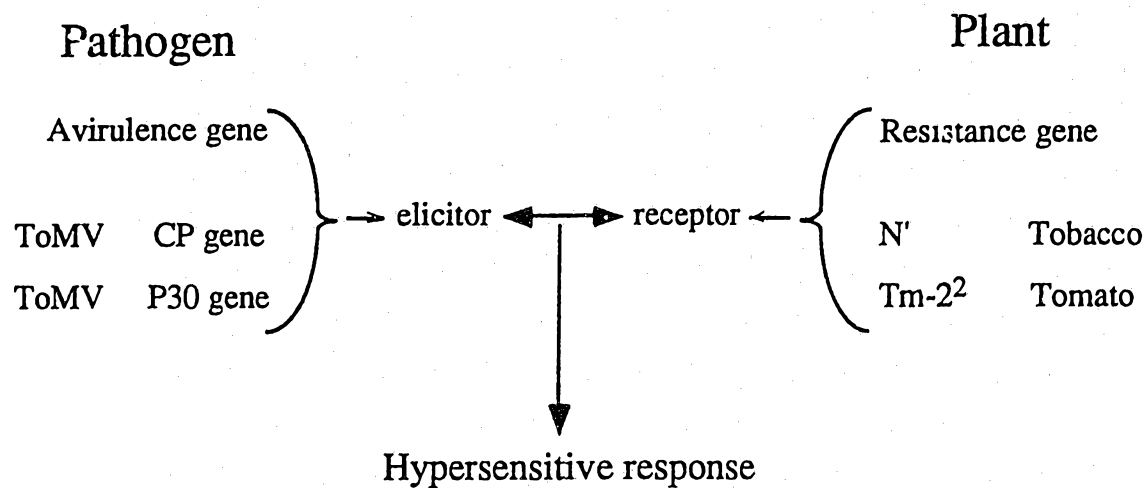


Figure 2.

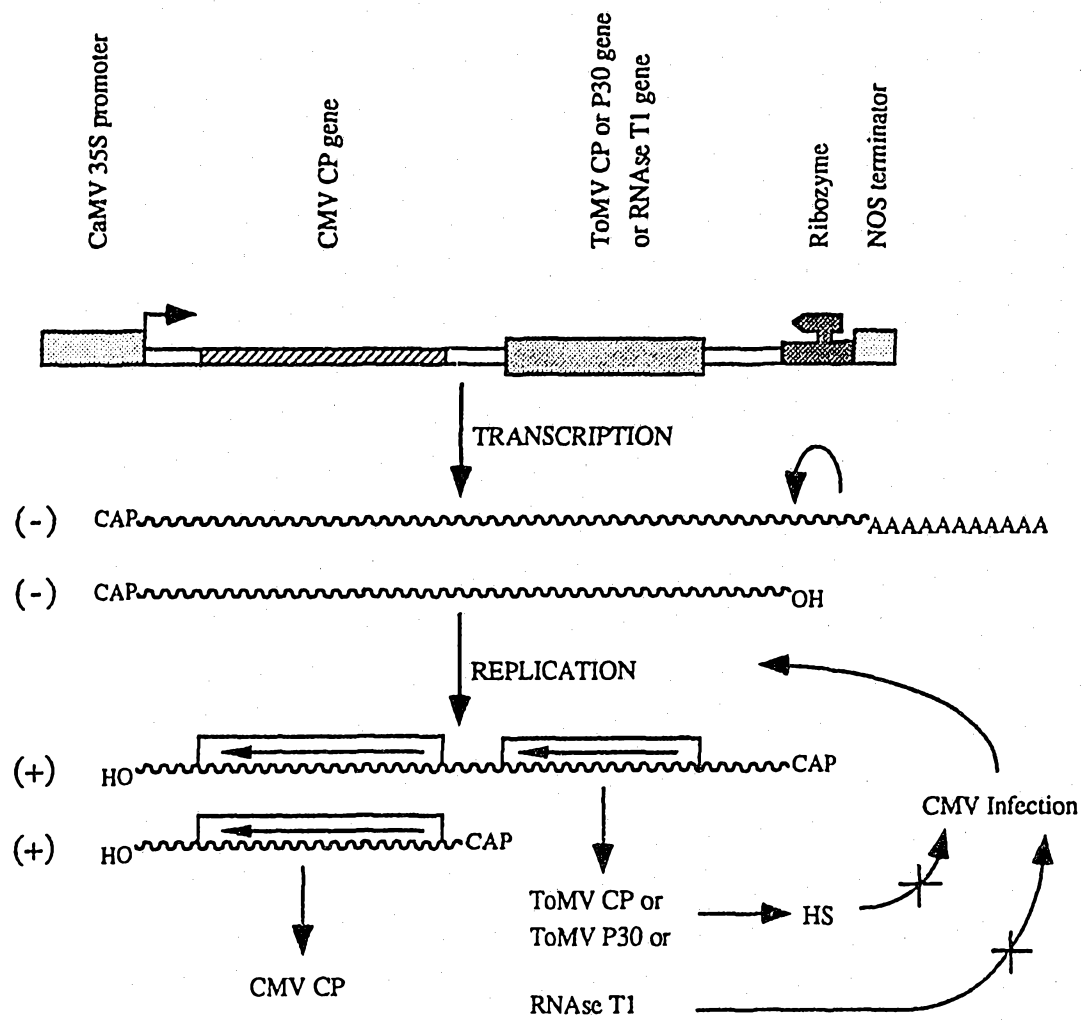
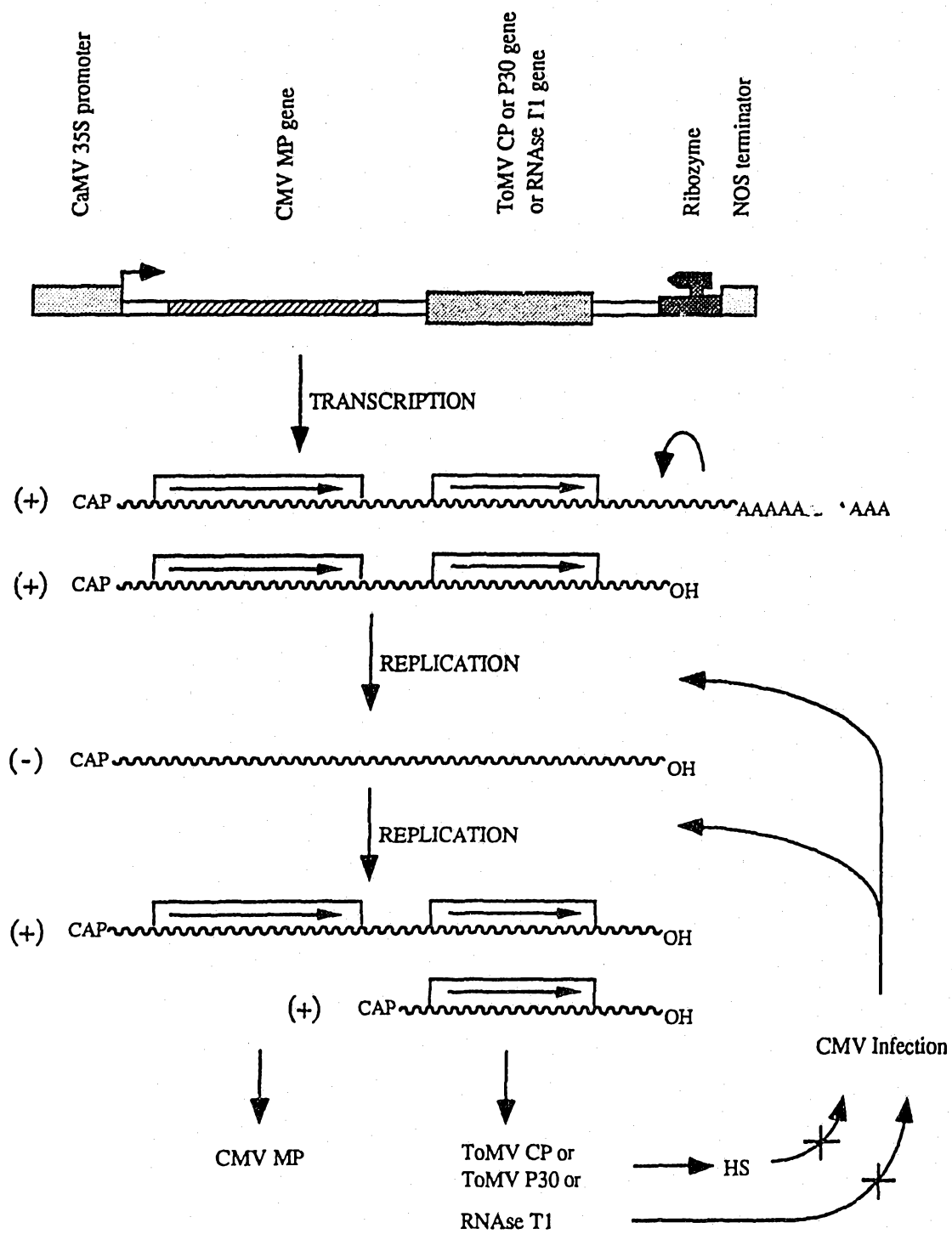


Figure 3



INTERNATIONAL SEARCH REPORT

 International application No
 PCT/EP 94/01817

 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 5 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N A01H

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 13994 (CSIRO) 19 September 1991 see page 7, line 33 - page 8, line 10 see page 14, line 30 - line 35 ---	1,7,8, 10-14
X	CHEMICAL ABSTRACTS, vol. 113, no. 15, 1990, Columbus, Ohio, US; abstract no. 127723, JUN, W. 'Preparation of transgenic plants for control of virosis' see abstract	1,7,8, 10-14
Y	& CN,A,1 033 645 (CHINESE ACADEMY OF SCIENCES) 5 July 1989 ---	9
Y	EP,A,0 298 918 (CIBA-GEIGY) 11 January 1989 see claim 14 --- -/--	9

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

11 October 1994

Date of mailing of the international search report

16. 11. 94

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 Fax (+31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01817

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 479 180 (HOECHST) 8 April 1992 see the whole document ----	1,7,8, 10-14
X	STADLER GENETICS SYMPOSIA SERIES: GENE MANIPULATION IN PLANT IMPROVEMENT, II. EDITED BY J. PERRY GUSTAFSON, 1990 pages 313 - 330 YOUNG, M., ET AL. 'Using plant virus and related RNA sequences to control gene expression' see page 318 - page 319 ----	1,7,8, 10-14
X	EP,A,0 425 004 (AVEVE) 2 May 1991 see page 7, line 15 - line 24; example 1 ----	1,14
X	AU,B,7 195 191 (NIHON NOHYAKU) 12 March 1992 see page 24, line 4 - line 6 ----	1,7,9,14
P,X	EP,A,0 573 767 (NIHON NOHYAKU) 15 December 1993 see page 9, line 20 - line 21 ----	1
A	BIOLOGICAL ABSTRACTS, vol. 92 1991, Philadelphia, PA, US; abstract no. 129186, CULVER, J.N., ET AL. 'Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic Nicotiana sylvestris' see abstract & MOL. PLANT MICROBE INTERACT., vol.4, no.5, 1991 pages 458 - 463 ----	9
A	BIOLOGICAL ABSTRACTS, vol. 94 1992, Philadelphia, PA, US; abstract no. 89218, PFITZNER, U.M., ET AL. 'Expression of a viral avirulence gene in transgenic plants is sufficient to induce the hypersensitive defense reaction' see abstract & MOL. PLANT MICROBE INTERACT., vol.5, no.4, 1992 pages 318 - 321 -----	9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP 94/01817

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP-A- 5260971	12-10-93

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		NL-A- 9001711	01-05-91
		CA-A- 2026703	04-04-91
		JP-A- 3280883	11-12-91

AU-B-7195191		NONE	

EP A-0573767	15-12-93	AU-B- 3824893	04-11-93
		JP-A- 6046874	22-02-94
