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Cervone et al.

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(54) **FUSION PROTEIN AND TRANSGENIC PLANT EXPRESSING SAID PROTEIN**

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PCT Pub. Date: **Jun. 30, 2016**

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A01N 25/10	(2006.01)
C07K 14/195	(2006.01)
C07K 14/37	(2006.01)
C07K 14/435	(2006.01)
C12N 15/82	(2006.01)
C07K 14/415	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/62*** (2013.01); ***A01N 25/10*** (2013.01); ***C07K 14/195*** (2013.01); ***C07K 14/37*** (2013.01); ***C07K 14/415*** (2013.01); ***C07K 14/43563*** (2013.01); ***C12N 15/8279*** (2013.01); ***C12N 15/8281*** (2013.01); ***C12N 15/8282*** (2013.01); ***C12N 15/8286*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention concerns a nucleic acid molecule capable of expressing, in at least one plant tissue, a chimeric protein comprising a polygalacturonase (PG) of fungal, bacterial or insect origin and a plant polygalacturonase inhibitor protein (PGIP) plant capable of inhibiting said PG. The present invention also relates to transgenic plants that express said chimeric protein.

11 Claims, 5 Drawing Sheets

Specification includes a Sequence Listing.

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FIG. 1A

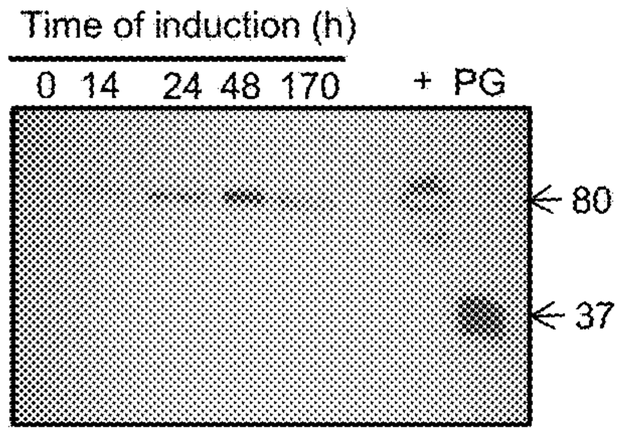


FIG. 1B

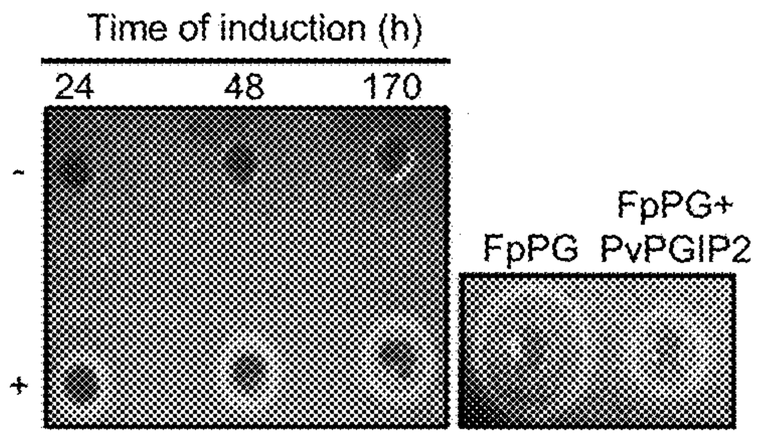


FIG. 1C

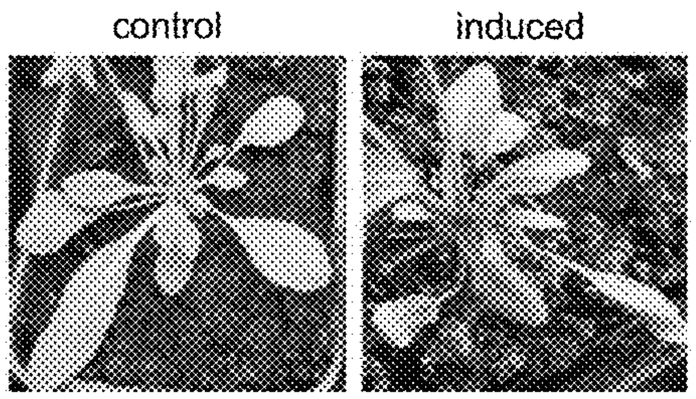


FIG. 1D

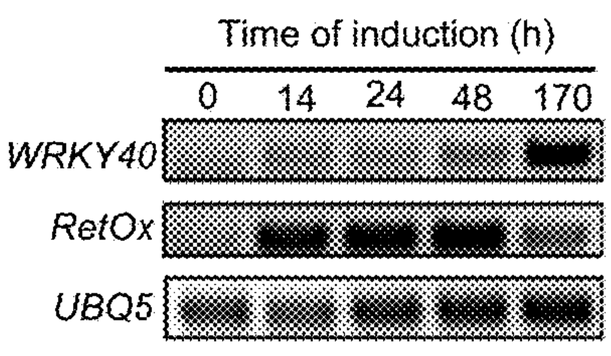


FIG. 1E

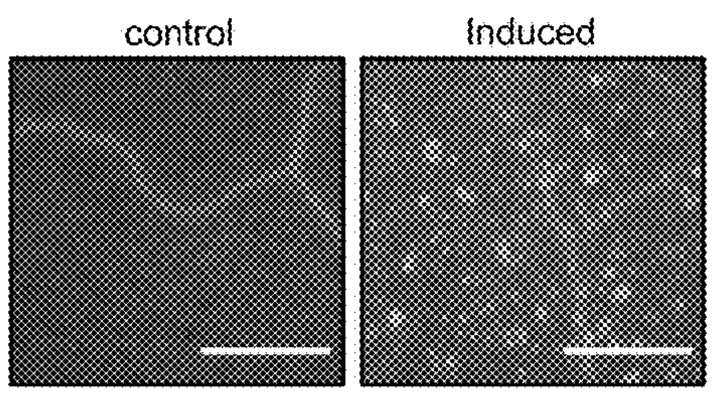


FIG. 1F

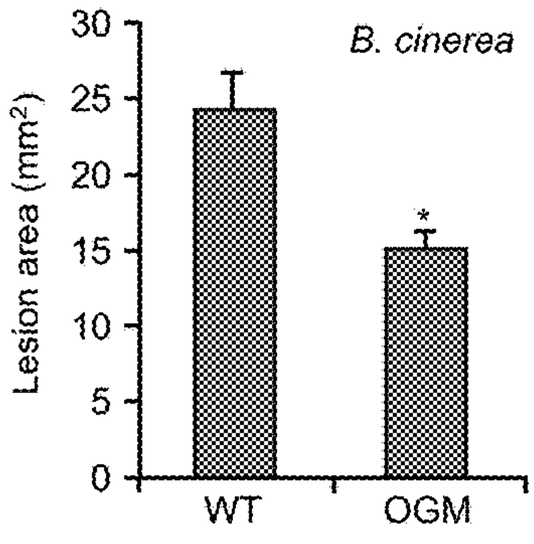


FIG. 1G

FIG. 2A

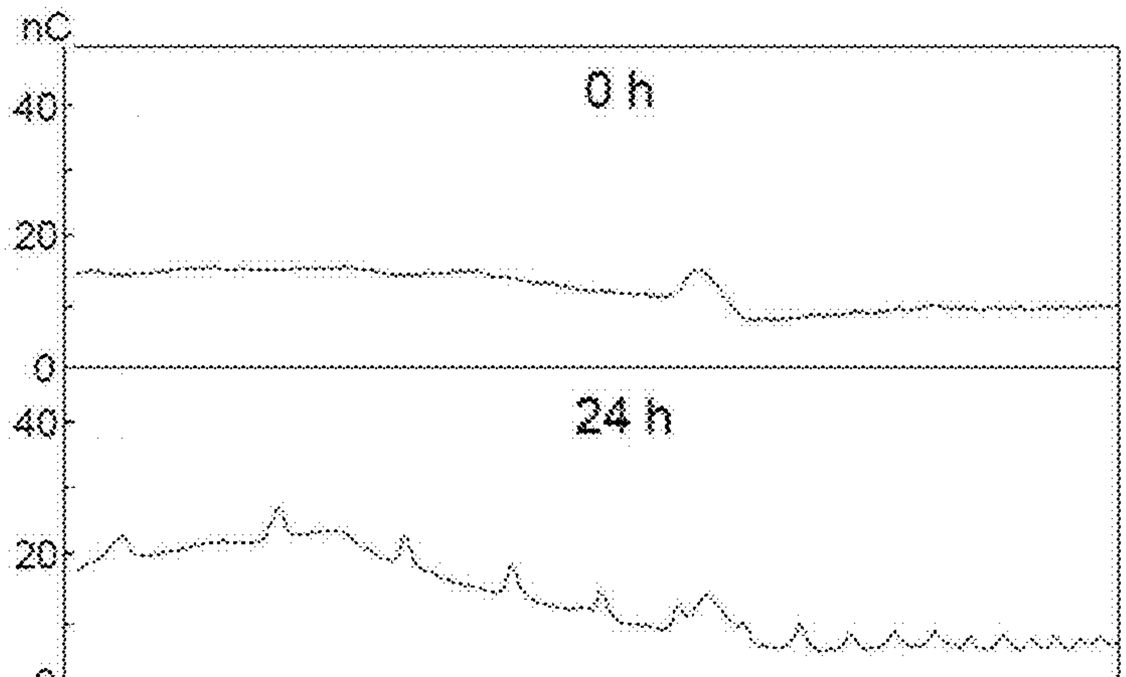


FIG. 2B

FIG. 2C

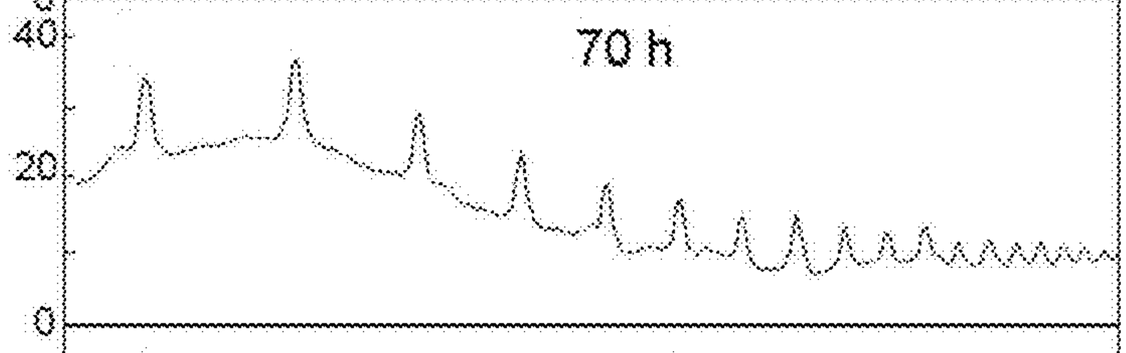


FIG. 2D

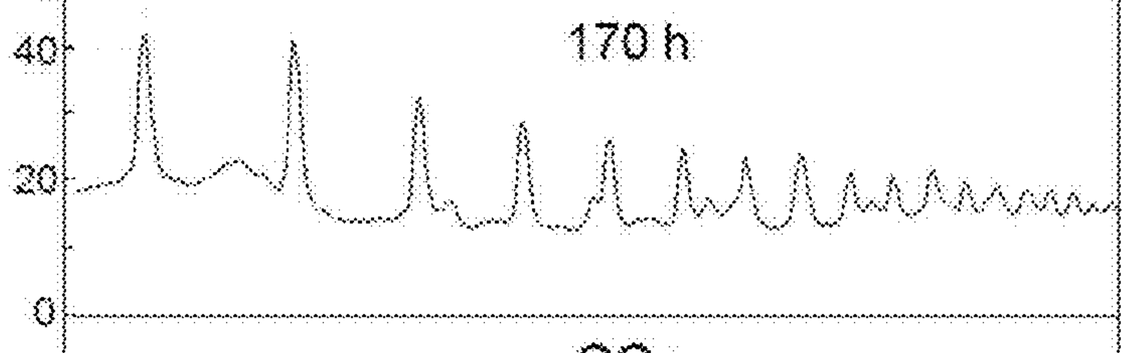
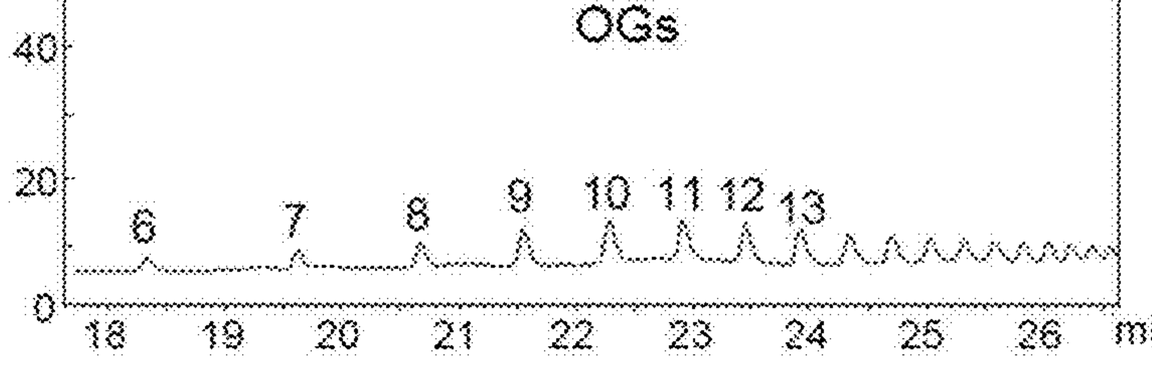


FIG. 2E



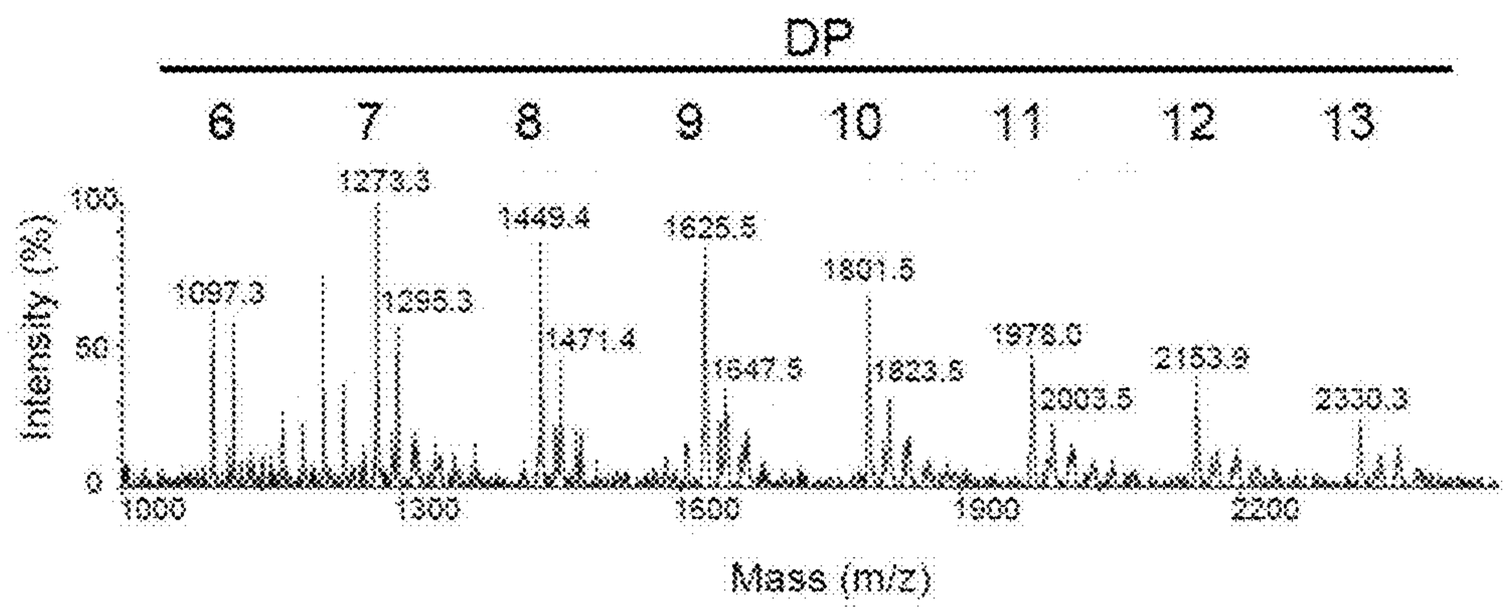


FIG. 2F

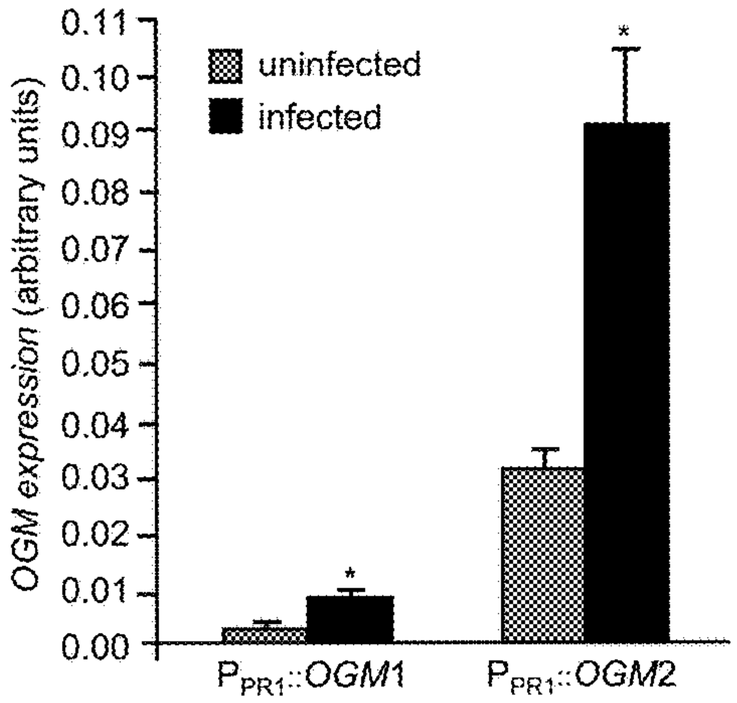


FIG. 3A

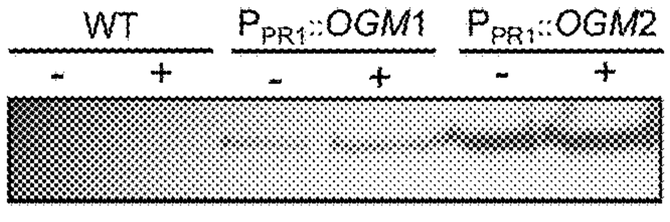


FIG. 3B

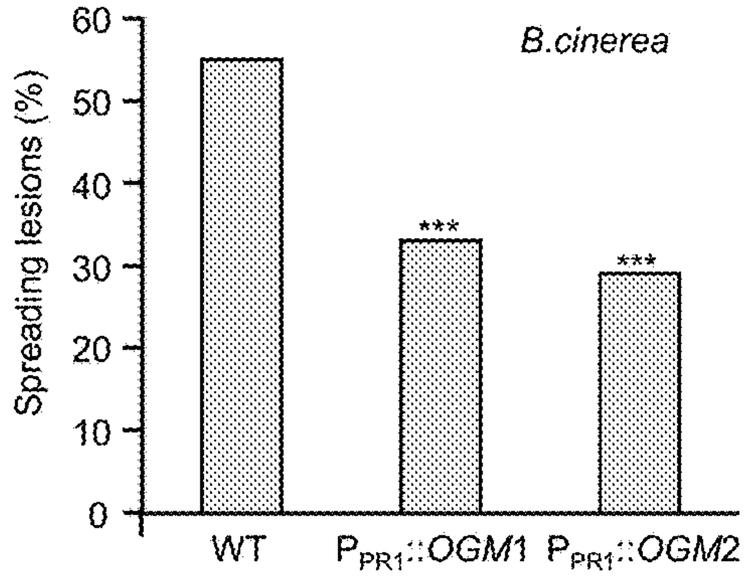


FIG. 3C

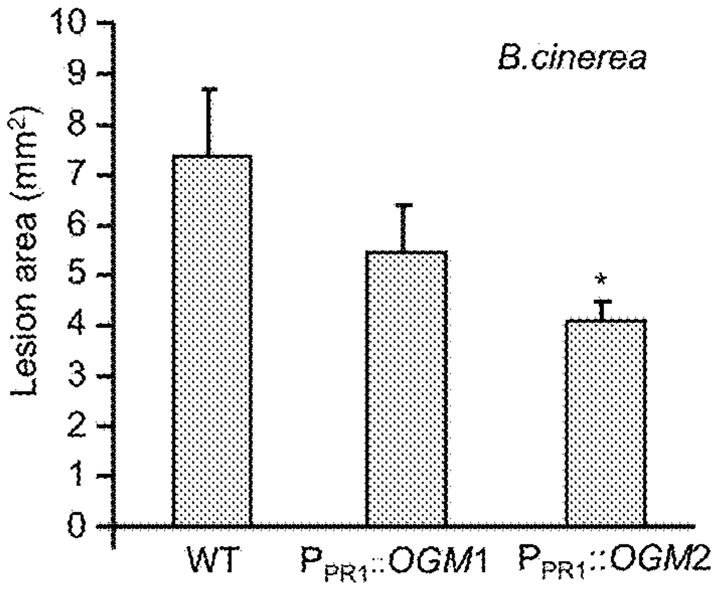


FIG. 3D

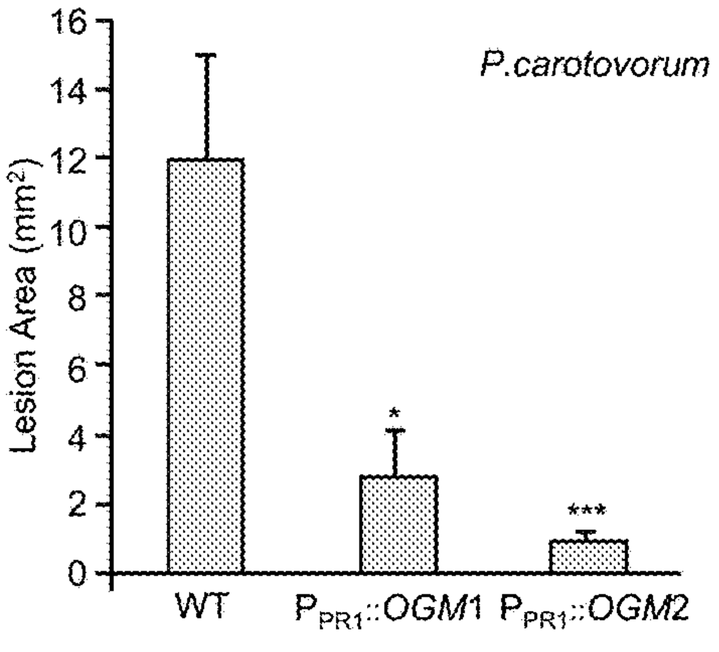


FIG. 3E

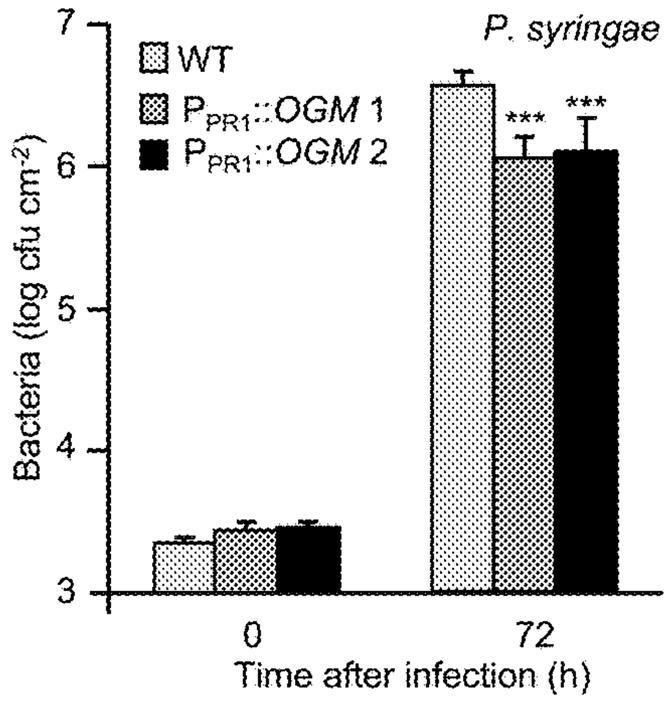


FIG. 3F

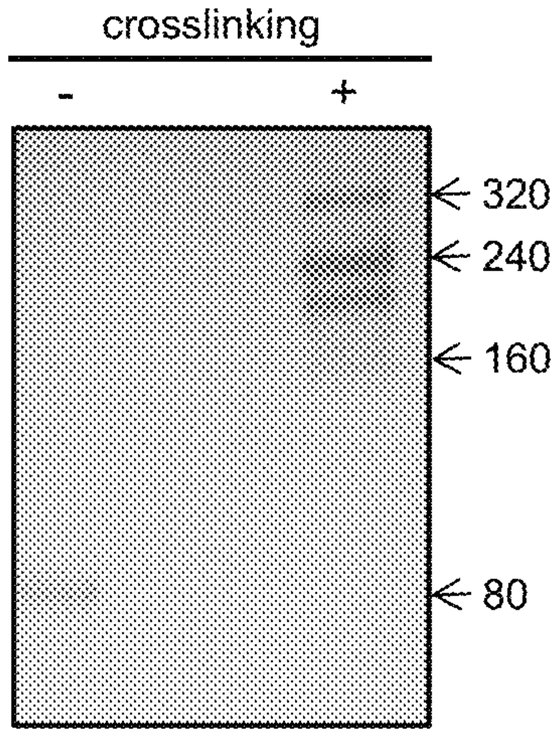


FIG. 4A

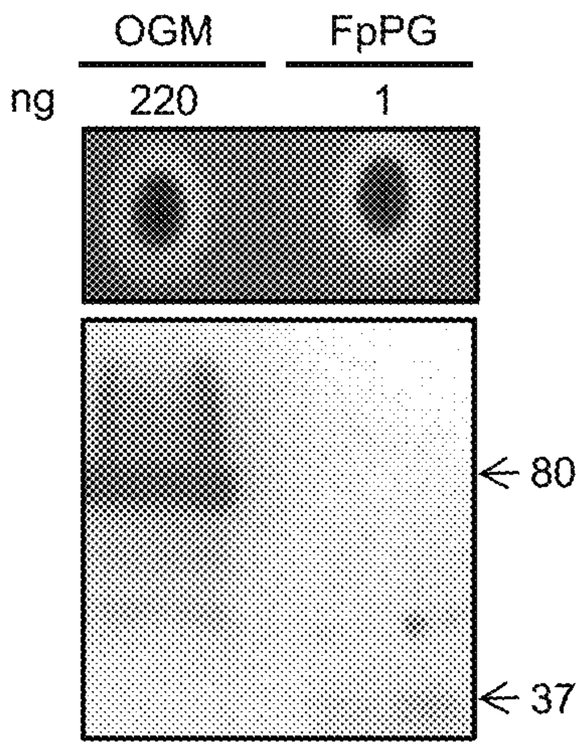


FIG. 4B

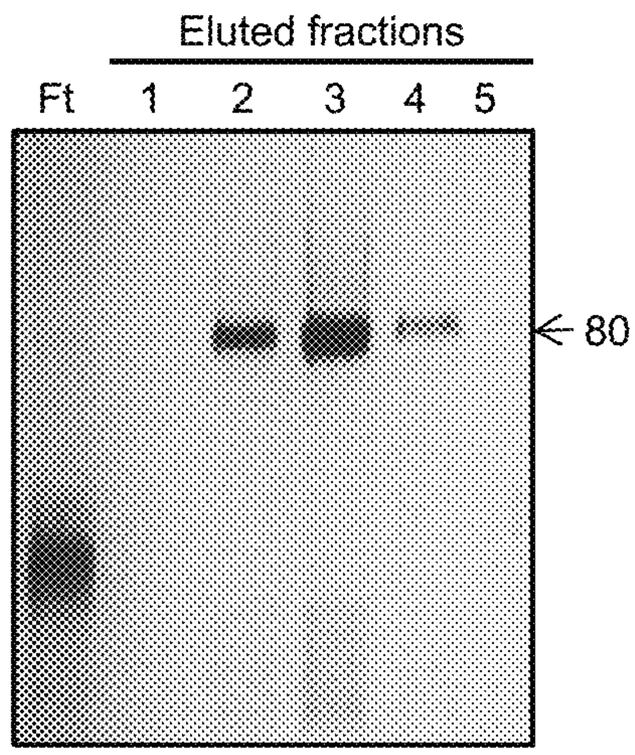


FIG. 4C

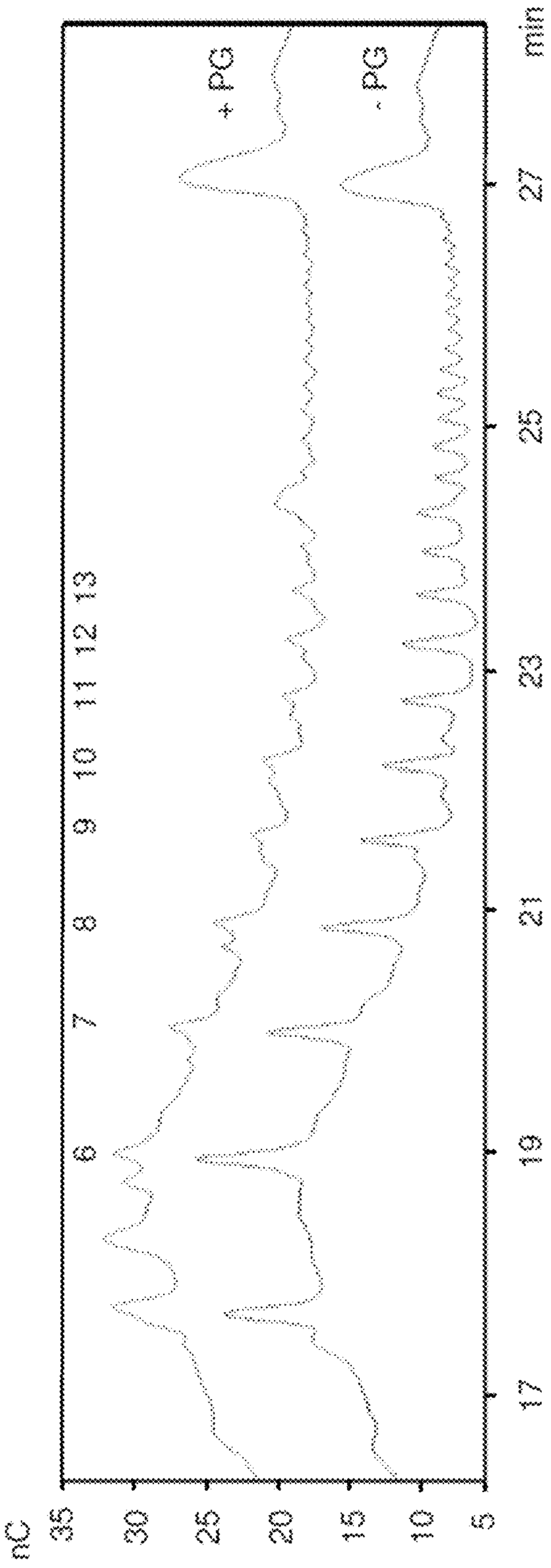


FIG. 5

FUSION PROTEIN AND TRANSGENIC PLANT EXPRESSING SAID PROTEIN

RELATED CASES

This application is the national stage entry under 35 U.S.C. § 371 of International Patent Application No. PCT/EP2015/081017, filed on Dec. 22, 2015, which claims the benefit of Italian Patent Application No. RM2014A000748, filed on Dec. 23, 2014, the entirety of each of which is incorporated herein by reference.

TECHNICAL FIELD

The present invention concerns a nucleic acid molecule capable of expressing, in at least one plant tissue, a chimeric protein comprising a polygalacturonase (PG) of fungal, bacterial or insect origin and a plant polygalacturonase inhibitor protein (PGIP) capable of inhibiting said PG. The present invention also relates to transgenic plants that express said chimeric protein.

PRIOR ART

Plant immunity is mediated not only by pathogen-derived molecules, called microbe-associated molecular patterns (MAMPs) (1), but also by endogenous molecules referred to as damage-associated molecular patterns (DAMPs), which are released by the host cell during a microbial infection (2-4).

Oligogalacturonides (OGs), oligomers of α-1,4-galacturonic acid released by the plant cell wall following a partial hydrolysis of homogalacturonan (HG), which is the main constituent of pectin, are the best characterised of all DAMPs (3).

Most information on OGs has been obtained from in vitro experiments using exogenous treatments; direct evidence of their accumulation and their function in plants has to date been lacking.

In the last 30 years evidence has been provided of the fact that OGs applied from the outside are capable of activating defence responses in plant tissues (2,3,5). It has been speculated that the enzymatic degradation of homogalacturonan, which takes place during microbial infections, leads to a OG-mediated defence response of the plant against pathogens. It has been demonstrated in vitro that the interaction between polygalacturonase (PG) and the polygalacturonase inhibitor protein (PGIP), located in the cell wall, can favour the accumulation of OGs with eliciting activity (4,6). The use of PGIP has since been tested in transgenic plants (7-9), where the resistance provided by the inhibitor against a fungus is dependent on the specificity of recognition of the inhibitor, which enables it to inhibit only some of the various existing fungal polygalacturonases (10,11).

U.S. Pat. No. 5,569,830 relates to nucleotide sequences encoding plant polygalacturonase inhibitor proteins (PGIP) which inhibit the activity of fungal polygalacturonases. Transgenic plants expressing a heterologous PGIP show an increased resistance to the fungi that normally infect plants.

Patent application EP0577252 relates to a method of creating a transgenic tomato containing a lowered level of the isoform 1 of polygalacturonase. The DNA sequence encoding at least a portion of the polygalacturonase beta-subunit which is sufficient to hybridise effectively to the mRNA for the polygalacturonase beta-subunit in vivo is used to create a construct in which the cDNA is positioned

in such a way as to produce the antisense version of the polygalacturonase beta-subunit message.

Moreover, the hypothesis that PGIP favors the production of OGs in vivo and that these molecules in turn act as defence signals during an infection has never been proven (Benedetti et al., Proceedings of the National Academy of Sciences, Vol. 112, no. 17, 2015).

Further, plants simultaneously expressing the PGII of *Aspergillus niger* and the PGIP2 of *Phaseolus vulgaris*, which is able to inhibit the PGII of *A. niger*, obtained by crossing two transgenic plants separately expressing either PG or PGIP, do not allow the production of OG in vivo (Ferrari S, Galletti R, Pontiggia D, Manfredini C, Lionetti V, Bellincampi D, Cervone F, De Lorenzo G: Transgenic expression of a fungal endo-polygalacturonase increases plant resistance to pathogens and reduces auxin sensitivity. Plant Physiol 2008, 146:669-681).

The basic concept is founded on evidence deriving from the use of OGs obtained in vitro from commercial pectins originating from tissues of different species of plants. OGs with a degree of polymerisation (DP) of between 10 and 15 activate a wide range of defence responses, such as the accumulation of phytoalexins (12-14), glucanase and chitinase (15, 16), as well as the expression of genes correlated with defence (17,18) and the production of reactive oxygen species (19, 20). In the model plant *Arabidopsis*, OGs are perceived by the wall-associated receptor kinase WAK1 (21, 22), activate gene expression independent of the signalling pathway which involves ethylene, salicylic acid and jasmonic acid (23) and activate the phosphorylation of the MAP kinases AtMPK3 and AtMPK6 (24). OGs can moreover induce a strong "oxidative burst" mediated by NADPH oxidase AtRbohD, which is also partly involved in the consequent accumulation of callose in the cell wall (20). Similarly, in the cells of mammals, DAMP signals deriving from the degradation of hyaluronan in the extracellular matrix activate inflammatory responses through the kinase receptors TLR2 and TLR4, which are also required for the perception of MAMPs (25). Therefore, MAMPs and DAMPs, notwithstanding their origin and distinctive characteristics, are functionally similar both in plants and animals (26).

DETAILED DESCRIPTION OF THE INVENTION

The authors have demonstrated that *Arabidopsis* plants expressing a chimeric protein obtained from the fusion between a fungal PG and a PGIP accumulate OGs in plant tissues and hence activate the plant defense responses. Furthermore, plants expressing this fusion protein (called OG-machine, initials OGM), under the control of a pathogen-inducible promoter, have an increased resistance against pathogenic microorganisms such as fungi and bacteria. The present data demonstrate that it is possible to engineer the release of DAMPs so as to be able to induce plant immunity in vivo. OGMs and DAMPs are thus powerful tools capable of providing protection to the plant against pathogens.

It is therefore an object of the present invention a nucleic acid molecule coding for a chimeric protein comprising:

a) an amino acid sequence with a polygalacturonase inhibitor (PGIP) activity of plant origin,

b) an amino acid sequence with a polygalacturonase (PG) activity as in a) of fungal, bacterial or insect origin.

Preferably, said chimeric protein comprises the sequence a) at the N-terminal portion and the sequence b) at the C-terminal portion.

In a preferred embodiment of the invention, the nucleic acid molecule codes for a chimeric protein wherein the amino acid sequence with the PGIP activity comprises a sequence selected from the group consisting of:

the sequence of PGIP2 of *Phaseolus vulgari* (Pv PGIP2) comprising the sequence SEQ ID NO:4, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity, a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of PGIP1 of *Phaseolus vulgari* (Pv PGIP1) comprising the sequence SEQ ID NO:23, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of PGIP3 of *Phaseolus vulgari* (Pv PGIP3) comprising the sequence SEQ ID NO:25, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of the PGIP of *Malus domestica* comprising the sequence SEQ ID NO: 26, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof or functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of PGIP1 of *Vitis vinifera* comprising the sequence SEQ ID NO: 28, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof or functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof; and

the sequence of PGIP1 or PGIP2 of *Arabidopsis thaliana* comprising respectively the sequence SEQ ID NO:30 or 31, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof.

In a more preferred embodiment of the invention, the amino acid sequence with the PGIP activity comprises the sequence of PGIP2 of *Phaseolus vulgaris* (Pv PGIP2) comprising or having essentially the sequence SEQ ID NO:4, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or isoforms thereof.

In a preferred embodiment of the invention, the nucleic acid molecule codes for a chimeric protein wherein the amino acid sequence with the PG activity comprises a sequence selected from the group consisting of:

the sequence of PG of *Fusarium phyllophilum* (FpPG) comprising the sequence SEQ ID NO:2 or SEQ ID NO:22, or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof or functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of PG2 of *Aspergillus niger* comprising the sequence SEQ ID NO:24 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof;

the sequence of the PG of *Colletotrichum lupini* comprising the sequence SEQ ID NO:27 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof or a functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof; and

the sequence of BcPG2 of *Botrytis cinerea* comprising the sequence SEQ ID NO:29 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof or functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof.

In a more preferred embodiment of the invention, the amino acid sequence with the PG activity comprises the sequence of PG of *Fusarium phyllophilum* (FpPG) comprising or having essentially the sequence SEQ ID NO:2, or a functional fragment of the same responsible for the polygalacturonase activity or isoforms thereof.

In a preferred embodiment of the invention, the nucleic acid molecule codes for a chimeric protein wherein the amino acid sequence with the PGIP activity comprises the sequence of PGIP2 of *Phaseolus vulgari* (Pv PGIP2) comprising the sequence SEQ ID NO:4, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof

and wherein the amino acid sequence with the PG activity comprises the sequence of PG of *Fusarium phyllophilum* (FpPG) comprising the sequence SEQ ID NO:2, or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof.

In a more preferred embodiment of the invention, the nucleic acid molecule codes for a chimeric protein, comprising:

a) the SEQ ID NO:4 and

b) the SEQ ID NO:2.

Preferably, the functional fragment of the sequence SEQ ID NO:4, has essentially the sequence aa. 30-aa. 342 of SEQ ID NO:4.

In an alternative preferred embodiment, the amino acid sequence with the PGIP activity comprises the sequence of PVPGIP1 of *Phaseolus vulgaris* comprising the sequence SEQ ID NO:23 or the sequence of PGIP2 of *Phaseolus vulgari* (Pv PGIP2) comprising the sequence SEQ ID NO:4, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof and wherein the amino acid sequence with the PG activity comprises the sequence of PG2 of *Aspergillus niger* comprising the sequence SEQ ID NO:24 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof.

In an alternative preferred embodiment, the amino acid sequence with the PGIP activity comprises the sequence of the PGIP of *Malus domestica* comprising the sequence SEQ ID NO:26, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof and wherein the amino acid sequence with the PG activity comprises the sequence of the PG of *Colletotrichum lupini* comprising the sequence SEQ ID NO:27 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof.

In a further preferred embodiment of the invention, the amino acid sequence with the PGIP activity comprises the sequence of PGIP1 of *Vitis vinifera* comprising the sequence SEQ ID NO:28, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof and wherein the amino acid sequence with the PG activity comprises the sequence of BcPG2 of *Botrytis cinerea* comprising the sequence SEQ ID NO: 29 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof.

In another preferred embodiment of the invention, the amino acid sequence with the PGIP activity comprises the sequence of PGIP1 or PGIP2 of *Arabidopsis thaliana* comprising the sequence SEQ ID NO:30 or 31 respectively, or a functional fragment of the same responsible for the polygalacturonase inhibitor activity or a isoform thereof and wherein the amino acid sequence with the PG activity comprises the sequence of BcPG2 of *Botrytis cinerea* comprising the sequence SEQ ID NO: 29 or a functional fragment of the same responsible for the polygalacturonase activity or a isoform thereof.

Preferably, the nucleic acid molecule according to the invention comprises a region coding for a linker, preferably comprised between the sequence coding for the amino acid sequence with PGIP activity and the sequence coding for the amino acid sequence with PG activity.

More preferably, said linker is of sequence Ala, Ala, Ala.

Preferably, the nucleic acid molecule according to the invention comprises a region coding for a signal peptide, preferably derived from bean or yeast.

In a preferred embodiment, the nucleic acid molecule to the invention comprises the nucleotide sequence having essentially the SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9.

Preferably, the nucleic acid molecule as above defined further comprises a promoter which is active in plants. Said promoter is preferably pathogen inducible, more preferably it is the promoter which regulates the expression of PR-1 gene of *Arabidopsis* (PPR-1) (PPR-1 [Genbank: Accession number: CP002685.1, from 6242431 bp to 6243722 bp]). Preferably, said promoter comprises or has essentially the sequence nt.1-1291 of the SEQ ID NO: 9. Other preferred promoters are e.g.: promoter which regulates the expression of VSR gene of *Arabidopsis* (PVSR) (PVSR [Genbank: Accession number: CP002684.1, from 10996136 bp to 10997274 bp]), promoter which regulates the expression of PBS2/RAR1 gene of *Arabidopsis* (PPBS2/RAR1) (PPBS2/RAR1 [Genbank: Accession number: CP002688.1, from 21003143 bp to 21004278 bp]).

Another object of the invention is an expression vector comprising the nucleic acid molecule as above defined. In said expression vector, the nucleic acid molecule is preferably under the control of a promoter which is active in plants. Said promoter is preferably pathogen inducible, more preferably it is the promoter which regulates the expression of PR-1 gene of *Arabidopsis* (PPR-1) (PPR-1 [Genbank: Accession number: CP002685.1, from 6242431 bp to 6243722 bp]). Preferably, said promoter comprises or has essentially the sequence nt.1-1291 of the SEQ ID NO: 9.

Other preferred promoters are e.g.: promoter which regulates the expression of VSR gene of *Arabidopsis* (PVSR) (PVSR [Genbank: Accession number: CP002684.1, from 10996136 bp to 10997274 bp]), promoter which regulates the expression of PBS2/RAR1 gene of *Arabidopsis* (PPBS2/RAR1) (PPBS2/RAR1 [Genbank: Accession number: CP002688.1, from 21003143 bp to 21004278 bp]).

Another object of the invention is the use of the vector as described above or of the nucleic acid molecule as described above for producing transgenic plants or transformed plant tissues or transformed plant cells.

A further object of the invention is a transgenic plant obtainable through the use as described above, or parts thereof.

Another object of the invention is a transgenic plant, or parts thereof, comprising the nucleic acid molecule as described above and/or expressing the chimeric protein or functional fragments thereof according to the invention.

Another object of the invention are the seeds of the transgenic plant of the invention.

Another object of the invention is a chimeric protein, or a functional fragment of the same, as described above.

A further object of the invention is a chimeric protein, or a functional fragment of the same, coded by the nucleic acid molecule as described above.

Preferably, said chimeric protein comprises the amino acid sequence having essentially the SEQ ID NO: 6 or the SEQ ID NO: 8 or functional fragment or equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue thereof.

Another object of the invention is a genetically engineered host cell comprising the nucleic acid molecule as described above or the vector as described above and/or expressing the chimeric protein as described above.

The functional fragment of the amino acid sequence with the PGIP activity can comprise, for example, at least 20, 25, 35, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330 or 340 aa of the SEQ ID NO: 4, 23, 25, 26, 28, 30 or 31.

The amino acid sequence with the PG activity comprising the sequence of PG of *Fusarium phyllophilum* (FpPG) can alternatively have the sequence of NCBI Uniprot Accession Number: Q07181.1:

(SEQ ID NO: 22)

ORIGIN

```
  1    mvrnivsrlc sqlfalpsss lqerdpcsvt eysglatavs scknivlngf qvptgkqldi

 61    sslqudstvt fkgtttfatt adndfnpivi sgsnititga sghvidgngq aywdgkgsns

121    nsnqkpdhfi vvqkttgnsk itnlniqnwp vhcfditgss qltisglild nragdkpnak

181    sgslpaahnt dgfdisssdh vtldnnhvyn qddcvavtsg trtiwsnmyc sgghglsigs

241    vggksdnvvd gvcgflssqw nsqngcriks nsgatgtinn vtyqniaitn istygvdvqq

301    dyinggptgk ptngvkisni kfikvtgtva ssaqdwfilc gdgscsgftf sgnaitgggk

361    tssenyptnt cps.
//
```

The functional fragment of the amino acid sequence with the PG activity can comprise, for example, at least 20, 25, 35, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330 or 340 aa of the SEQ ID NO: 2, 22, 24, 27 or 29.

Preferably, the nucleic acid molecule according to the invention comprises the nucleotide sequence having essentially the SEQ ID NO:3 and/or the SEQ ID NO:1.

The above described linker is preferably 2-10 amino acid long, more preferably 3-8 amino acid long, even more preferably it is 3 amino acid long.

Preferably, said linker comprises hydrophobic aminoacids. More preferably, said linker is of the sequence Ala, Ala, Ala.

The linker as above defined may be selected by the skilled in the art according to its properties. In particular, said linker should be of a proper length which allows to avoid the proteolytic cleavage of the chimeric protein. Said linker allows the equimolar production of the amino acid sequence with PGIP activity and of the amino acid sequence with PG activity. The linker also allows the intermolecular interaction between the PGIP and the PG moieties, while not permitting intramolecular enzyme-inhibitor interactions.

The chimeric protein according to the invention, is preferably expressed under the control of a promoter that is non constitutive and which is preferably active only during the infection.

Said promoter is preferably the promoter which regulates the expression of the gene PR-1 of *Arabidopsis* (PPR-1 [Genbank: Accession number: CP002685.1, from 6242431 bp to 6243722 bp]). Preferably, said promoter comprises or has essentially the sequence nt.1-1291 of the SEQ ID NO: 9.

The nucleic acid of the invention allows to obtain the expression of both PGIP and PG simultaneously and in equimolar amounts.

The transgenic plants according to the invention thus accumulate equimolar levels of PG and PGIP.

As used here, the term "nucleic acid" refers to RNA or DNA, preferably DNA. Said DNA can be double-stranded or single-stranded. The term also includes the complementary strand of the specified sequences. The nucleic acid molecule of the invention can also include additional coding sequences, such as a leader sequence or a pro-protein sequence, and/or additional non-coding sequences, such as UTR sequences.

The term "nucleic acid" can also refer to a "vector", such as, for example, an expression vector. The term "expression vector" comprises, for example, a plasmid, a viral particle, a phage, etc. Such vectors can include bacterial plasmids, phagic DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral DNA, such as vaccinia, adenovirus, fowl pox virus and pseudorabies virus. A large number of suitable vectors are known to the person skilled in the art and are commercially available.

The nucleic acid molecule, preferably the DNA sequence, in the vector is operatively linked to an appropriate expression control sequence (promoter) for direct mRNA synthesis. As examples representative of such promoters, one may mention a prokaryote or eukaryote promoter such as a CMV immediate-early promoter, HSV thymidine kinases, early and late SV40 and retrovirus LTR. The promoter is preferably the above defined promoter of PR-1. The expression vector also contains a ribosomal binding site for starting the translation and a transcription vector. The vector can also include sequences appropriate for the expression of amplification.

Furthermore, the vectors preferably contain one or more marker genes that can be selected to provide a phenotypic trait for the selection of the transformed host cell, e.g. dihydrofolate reductase or neomycin resistance for the culture of eukaryotic cells, or tetracycline or ampicillin resistance in *E. coli*.

As used here, the term "genetically engineered host cell" refers to host cells that have been transduced, transformed or transfected with the nucleic acid molecule or with the vector previously described.

As representative examples of appropriate host cells, one may mention bacterial cells such as *E. coli, Streptomyces* and *Salmonella typhimurium* cells, fungal cells such as yeast cells, insect cells such as Sf9 cells, animal cells such as CHO or COS cells, plant cells etc. The selection of an appropriate host is considered in the field of application by the person skilled in the art. Preferably, said host cell is a plant cell.

The nucleic acid molecule or vector previously described can be introduced into the host cell using methods that are well known to a person skilled in the art, e.g. transfection with calcium phosphate, DEAE-dextran mediated transfection, biolistic particle bombardment, transformation mediated by *Agrobacterium tumefaciens* or electroporation.

The nucleic acid molecule of the invention can comprise a region coding for a linker capable of favouring intra- or intermolecular interaction in the chimeric protein.

In the context of the present invention the terms "protein", "amino acid sequence with PGIP activity" or "amino acid sequence with PG activity" include:

i. the whole protein (for example pvPGIP2 [Uniprot Accession Number: P58822.1], or the SEQ ID NO:4, or FpPG [Uniprot Accession Number: Q07181.1] or the SEQ ID NO:2, or the other PGIPs and PGs above described), isoform, allelic variants and proteins coded by an orthologous gene of the same (for example, proteins coded by an orthologous gene of Pv PGIP2 or of SEQ ID NO:4, or proteins coded by an orthologous gene of FpPG or of SEQ ID NO:2 or of the other PGIPs and PGs above described);

ii. any functional fragment of the protein with an inhibiting activity of PG, in the case of PGIP protein fragments, or polygalacturonase activity, in the case of PG protein fragments;

iii. any equivalent, variant, mutant, functional derivative, synthetic or recombinant functional analogue, with PG-inhibiting activity in the case of equivalents, variants, mutants, functional derivatives, synthetic or recombinant functional analogues of the PGIP protein or of the amino acid sequence with PGIP activity (or of SEQ ID NO: 4 or of the sequences of the other PGIPs above described) or with polygalacturonase activity in the case of equivalents, variants, mutants, functional derivatives and synthetic or recombinant functional analogues of the PG protein or of the amino acid sequence with PG activity (or of SEQ ID NO: 2 or of the sequences of the other PGs above described).

In the context of the present invention, the term "functional fragment" of the chimeric protein as described above refers to fragments which, once expressed in a plant, plant tissue or plant cells are capable of inducing the release of oligogalacturonides (OGs). Said fragment may be long 100, 200, 250, 300, 350, 400, 450, 500, 550, 600 amino acids.

The term "chimeric protein" also includes its functional equivalent, variant, mutant, derivative, synthetic or recombinant functional analogue.

The terms "mutant" or "derivative" or "variant", as used in the context of the present invention can be intended as the substitution, deletion and/or addition of a single amino acid in the protein sequence. Preferably, the mutation of the protein sequence in the present invention is a substitution. The substitution can take place with a genetically coded amino acid or a non-genetically coded amino acid. Examples of non-genetically coded amino acids are homocysteine, hydroxyproline, omithin, hydroxylysine, citrulline, carnitine, etc.

As used here, the term "equivalent" means a peptide having at least one of the activities of the protein PGIP or PG or the same activity of the chimeric protein of the invention.

"Analogue" will be understood to mean a protein that exhibits some modifications relative to the proteins PG or PGIP. These modifications can be a deletion, a truncation, an extension, a chimeric fusion, and/or a mutation. Among the analogue proteins, those showing more than 80% of identity are preferred.

"Derivative" refers to any protein, possibly mutated, truncated, and/or extended, which was chemically modified or contains unusual amino acids.

As used here, the term "derivatives" refers also to proteins having a percentage of identity of at least 75% with the sequences disclosed in the present invention, e.g. with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO:8, preferably at least 85%, for example at least 90%, and more preferably at least 95%.

The chimeric protein of the invention, if required, can be modified in vitro and/or in vivo, for example by glycosylation, myristoylation, amidation, carboxylation or phosphorylation, and can be obtained, for example, by means of synthetic or recombinant techniques known in the art.

As used here, the term "orthologues" refers to genes in different species relative to the gene coding for the proteins PvPGIP, or of SEQ ID NO:4, or FpPG, or of SEQ ID NO:2 or relative to the gene for the proteins as above defined. As examples of such orthologues, one may mention the proteins corresponding to PGIP in *Arabidopsis thaliana, Nicotiana tabacum, Glycine max, Gossypium arboreum, Brassica napus, Vitis vinifera* and *Beta vulgaris* or PG in *Botrytis cinerea, Aspergillus niger, Colletotricum lupini, Fusarium oxysporum, Erwinia carotovora, Lygus ruguhpennis* and *Adelphocoris lineolatus*.

In further preferred embodiments, the nucleic acid molecule of the invention codes for a protein comprising the sequences of:

the isoform PVPGIP1 of *Phaseolus vulgaris* (NCBI Uniprot Accession Number P35334.1:

```
ORIGIN
  1    mtqfnlpvtm ssslsillvi lvslrtalse lcnpqdkqal lqikkdlgnp ttlsswlptt

 61    dccnrtwlgv lcdtdtqtyr vnnldlsghn lpkpypipss lanlpylnfl yigginnlvg

121    pippaiaklt alhylyitht nvsgaipdfl sqiktlvtld fsynalsgtl ppsisslpnl

181    ggitfdgnri sgaipdsygs fsklftamti srnrltgkip ptfanlnlaf vdlsrnmleg

241    dasvlfgsdk ntkkihlakn slafdlgkvg lsknlngldl rnnriygtlp qgltqlkflq

301    slnvsfnnlc geipqggnlk rfdvssyann kclcgsplps ct
//
(SEQ ID NO: 23))
and
```

the isoform PG2 of *Aspergillus niger* (Uniprot Accession Number P26214.1:

```
ORIGIN
  1    mhsfasllay glvagatfas aspieardsc tfttaaaaka gkakcstitl nnievpagtt

 61    ldltgltsgt kvifegtttf gyeewagpli smsgehitvt gasghlincd garwwdgkgt

121    sgkkkpkffy ahgldsssit glnikntplm afsvqandit ftdvtinnad gdtqgghntd

181    afdvgnsvgv niikpwvhnq ddclavnsge niwftggtci gghglsigsv gdrsnnvvkn

241    vtiehstvsn senavrikti sgatgsvsei tysnivmsgi sdygvviqqd yedgkptgkp

301    tngvtiqdvk lesvtgsvds gateiyllcg sgscsdwtwd dvkvtggkks tacknfpsva

361    sc
//
(SEQ ID NO: 24)) (10);
```

the PGIP of *Malus domestica* (NCBI Uniprot Accession Number P93270.1):

```
ORIGIN
  1    melkfsifls ltllfssvlk palsdlcnpd dkkvllqikk afgdpyvlts wksdtdccdw

 61    ycvtcdsttn rinsltifag qvsgqipalv gdlpyletle fhkqpnitgp iqpaiaklkg

121    lkflrlswtn lsgsvpdfls qlknitfldl sfnnitgaip sslsqlpnln alhldrnklt

181    ghipkslgqf ignvpdlyls hnqlsgnipt sfaqmdftsi dlsrnklegd asvifglnkt

241    tqivdlsrnl lefnlskvef ptsltsldin hnkiygsipv eftqlnfqfl nvsynrlcgq

301    ipvggklqsf deysyfhnrc lcgaplpsck
//
(SEQ ID NO: 26))
and
```

-continued

the PG of *Colletotrichum lupini* var *setosum* (NCBI Uniprot Accession Number A1E266.1):

```
ORIGIN
  1    mvssllalga laataiaapl darasctftd aaaaikgkas ctsiilngiv vpagttldmt

 61    glksgttvtf qgkttfgyke wegplisfsg tniningasg hsidcqgsrw wdskgsnggk

121    tkpkffyahs lkssnikgln vintpvqafs insattlgvy dviidnsagd sagghntdaf

181    dvgsstgvyi sganvknqdd clainsgtni tftggtcsgg hglsigsvgg rsdntvktvt

241    isnskivnsd ngvriktvsg atgsysgvty sgitlsniak ygivieqdye ngsptgtptn

301    gvpitgltls kitgsvassg tnvyilcasg acsnwkwsgv svtggkkstk csnipsgsga

361    ac
//
(SEQ ID NO: 27))
```

(Oelofse D, Dubery I A, Meyer R, Arendse M S, Gazendam I, Berger D K: Apple polygalacturonase inhibiting protein1 expressed in transgenic tobacco inhibits polygalacturonases from fungal pathogens of apple and the anthracnose pathogen of lupins. Phytochem 2006, 67:255-263.);

the isoform PGIP1 of *Vitis vinifera* (NCBI Uniprot Accession Number A7PW81.1):

```
ORIGIN
  1      metsklflls sslllvllat rpcpslserc npkdkkvllq ikkaldtpyi laswnpntdc

 61      cgwycvecdl tthrinslti fsgqlsgqip davgdlpfle tlifrklsnl tgqippaiak

121      lkhlkmvrls wtnlfgpvpa ffselknity ldlsfnnlsg pipgslslp nlgalhidrn

181      hltgpipdsf gkfagstpgl hlshnqlsgk ipysfrgfdp nvmdlsrnkl egdlsiffna

241      nkstqivdfs rnlfqfdlsr vefpksltsl dlshnkiags lpemmtsldl qfinvsynrl

301      cgkipvggkl qsfdydsyfh nrcicgaplq sck
//
(SEQ ID NO: 28))
```

and

the isoform BcPG2 of *Botrytis cinerea* (NCBI Uniprot Accession Number A4VB48.1):

```
ORIGIN
  1      mvhitslisf lastalvsaa pgsapadldr ragctfstaa taiaskttcs tiildsvvvp

 61      agttldltgl ktgtkvifqg tatfgysewe gplisisgqd ivvtgasgnk idgggarwwd

121      glgsnvsagk gkvkpkffsa hkltgsssit glnflnapvq cisiggsvgl slininidns

181      agdagnlghn tdafdinlsq nifisgaivk nqddcvavns gtnitftggn csgghglsig

241      svggrsgtga ndvkdvrfls stvqkstngv rvktvsdtkg svtgvtfqdi tligitgvgi

301      dvqqdyqngs ptgtptngvp itgltmnnvh gnviggqnty ilcancsgwt wnkvavtggt

361      vkkacagvpt gasc
//
(SEQ ID NO: 29))
```

(Joubert D A, Kars I, Wagemakers L, Bergmann C, Kemp G, Vivier M A, van Kan J A L: A polygalacturonase-inhibiting protein from grapevine reduces the symptoms of the endopolygalacturonase BcPG2 from *Botrytis cinerea* in *Nicotiana benthamiana* leaves without any evidence for in vitro interaction. Mol Plant-Microbe Interact 2007, 20:392-402);

The isoforms PGIP1 or PGIP2 of *Arabidopsis thaliana* (NCBI Uniprot Accession Number Q9M5J9.1 and Q9M5J8.2, respectively]):

```
ORIGIN
  1      mdktatlcll flftflttcl skdlcnqndk ntllkikksl nnpyhlaswd pqtdccswyc

 61      lecgdatvnh rvtaltifsg qisgqipaev gdlpyletiv frklsnitgt iqptiaklkn

121      lrmlrlswtn ltgpipdfis qlknleflel sfndlsgsip sslstlpkil alelsrnklt
```

-continued

```
181     gsipesfgsf pgtvpdlrls hnqlsgpipk slgnidfnri dlsrnklqgd asmlfgsnkt

241     twsidlsrnm fqfdiskvdi pktlgildln hngitgnipv qwteaplqff nvsynklcgh

301     iptggklqtf dsysyfhnkc lcgapleick
//
(SEQ ID NO: 30)

ORIGIN
  1     mdktmtlfll lstllttsl akdlchkddk ttllkikksl nnpyhlaswd pktdccswyc

 61     lecgdatvnh rvtsliiqdg eisgqippev gdlpyltsli frkltnitgh iqptiaklkn

121     ltflrlswtn ltgpvpefls qlknleyidl sfndlsgsip sslsslrkle ylelsrnklt

181     gpipesfgtf sgkvpslfls hnqlsgtipk slgnpdfyri dlsrnklqgd asilfgakkt

241     twivdisrnm fqfdlskvkl aktlnnldmn hngitgsipa ewskayfqll nvsynrlcgr

301     ipkgeyiqrf dsysffhnkc lcgaplpsck
//
(SEQ ID NO: 31))
```

and the isoform BcPG2 of *Botrytis cinerea* (NCBI Uniprot Accession Number A4VB48.1) (SEQ ID NO:29)) (Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel F M, Dewdney J: Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiol 2007, 144:367-379)

or any other combinations of PG and PGIP known to the expert in the art.

In the context of the present invention fungal origin means that the protein is of e.g. a fungus included in the genera *Fusarium*, as e.g. *Fusarium oxysporum, Fusarium phyllophiliu, Aspergillus*, as e.g. *Aspergillus niger, Botrytis*, as e.g. *Botrytis cinerea, Colletotrichum*, as e.g. *Colletotrichum lupine.*

In the context of the present invention bacterial origin means that the protein is of e.g. a bacterium included in the genera *Erwinia*, as e.g. *Erwinia carotovora.*

```
A further isoform of PGIP included in the present invention is, for
example, PvPGIP3 (Uniprot Accession Number P58823.1:
ORIGIN:
  1     mtqfnipvtm ssslsiilvi lvslrtalse lcnpqdkqal lqikkdlgnp ttlsswlptt

 61     dccnrtwlgv lcdtdtqtyr vnnldlsghn lpkpypipss lanlpylnfl yigginnlvg

121     pippaiaklt qlhylyitht nvsgaipdfl sqiktlvtld fsynalsgtl ppsisslpnl

181     vgitfdgnri sgaipdsygs fsklftsmti srnrltgkip ptfanlnlaf vdlsrnmlqg

241     dasvlfgsdk ntqkihlakn sldfdlekvg lsknlngldl rnnriygtlp qgltqlkflh

301     slnvsfnnlc geipqggnlq rfdvsayann kclcgsplpa ct
//
(SEQ ID NO: 25)).
```

The nucleic acid molecule according to the invention also comprises sequences having 70%, 80%, 90%, 95% or 100% identity sequence with the nucleotide sequence of SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9.

The transgenic plants according to the invention are resistant to bacterial and/or fungal and/or insect infections, produced e.g. by *Botrytis cinerea, Pectobacterium carotovorum* and *Pseudomonas syringae* p.v. *tabaci* DC3000.

In the context of the present invention the "plant" may be e.g. a plant included in the genera *Arabidopsis*, as e.g. *Arabidopsis thaliana*, or *Phaseolus*, as e.g. *Phaseolus vulgaris*, or *Nicotiana*, as e.g. *Nicotiana tabacum*, or *Glycine*, as e.g. *Glycine max*, or *Gossypium*, as e.g. *Gossypium arboreum*, or *Brassica*, as e.g. *Brassica napus*, or *Vitis*, as e.g. *Vitis vinifera*, or *Beta*, as e.g. *Beta vulgaris*, or *Triticum*, as e.g. *Triticum aestivum*, or *Solanum*, as e.g. *Solanum lycopersicum, Solanum tuberosum* and *Solanum melongena* L., or *Musa*, as e.g. *Musa acuminata* and *Musa balbisiana*, or *Fragaria*, as e.g. *Fragaria vesca, Fragaria viridis* and *Fragaria moschata*, or *Oryza*, as e.g. *Oryza sativa*, or *Hordeum*, as e.g. *Hordeum vulgare*, or *Olea*, as e.g. *Olea europaea.*

In the context of the present invention bacterial origin means that the protein is of e.g. a insect included in the genera *Lygus*, as e.g. *Lygus rugulipennis*, and *Adelphocoris*, as e.g. *Adelphocoris lineolatus*.

SEQUENCES

NUCLEOTIDE SEQUENCE of *Fusarium phyllophilum* PG (cDNA without the sequence coding for the signal peptide and the proteolytic cleavage signal ["GAT" nucleotides])

(SEQ ID NO: 1)

```
CCCTGCTCCGTGACTGAGTACTCTGGCCTCGCCACCGCTGTCTCATCCT

GCAAAAACATCGTGCTCAACGGTTTCCAAGTCCCGACAGGCAAGCAACT

CGACCTATCCAGCCTCCAGAATGACTCGACCGTTACCTTCAAGGGCACG

ACCACTTTTGCCACCACTGCTGATAACGACTTTAATCCTATCGTCATTA

GTGGAAGTAACATCACTATCACTGGTGCATCTGGCCATGTCATTGATGG
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-continued

SEQUENCES

CAACGGTCAGGCGTACTGGGATGGCAAAGGTTCTAACAGCAATAGCAAC

CAAAAGCCCGATCACTTCATCGTTGTTCAGAAGACCACCGGCAACTCAA

AGATCACAAACCTAAATATCCAGAACTGGCCCGTTCACTGCTTCGACAT

TACAGGCAGCTCGCAATTGACCATCTCAGGGCTTATTCTTGATAACAGA

GCTGGCGACAAGCCTAACGCCAAGAGCGGTAGCTTGCCCGCTGCGCATA

ACACCGACGGTTTCGACATCTCGTCCAGTGACCACGTTACGCTGGATAA

CAATCATGTTTATAACCAAGATGATTGTGTTGCTGTTACTTCCGGTACA

AACATCGTCGTTTCTAACATGTATTGCTCCGGCGGCCATGGTCTTAGTA

TCGGATCTGTTGGTGGAAAGAGCGACAATGTCGTTGATGGTGTTCAGTT

CTTGAGCTCGCAGGTTGTGAACAGTCAGAATGGATGTCGCATCAAGTCC

AACTCTGGCGCAACTGGCACGATCAACAACGTCACCTACCAGAACATTG

CTCTCACCAACATCAGCACGTACGGTGTCGATGTTCAGCAGGACTATCT

CAACGGCGGCCCTACTGGAAAGCCGACCAACGGAGTCAAGATCAGCAAC

ATCAAGTTCATCAAGGTCACTGGCACTGTGGCTAGCTCTGCCCAGGATT

GGTTTATTCTGTGTGGTGATGGTAGCTGCTCTGGATTTACCTTCTCTGG

AAACGCTATTACTGGTGGTGGCAAGACTAGCAGCTGCAACTATCCTACC

AACACTTGCCCCAGCTAG

AMINO ACID Sequence of *Fusarium phyllophilum* PG (without the amino acid sequence coding for the signal peptide and amino acid D, proteolytic cleavage signal) (SEQ ID NO: 2, corresponding to region aa. 26-aa. 373 of the sequence with Accession No. NCBI Q07181.1)

ORIGIN

```
  1      PCSVTEYSGL ATAVSSCKNI

 21      VLNGFQVPTG KQLDLSSLQN

 41      DSTVTFKGTT TFATTADNDF

 61      NPIVISGSNI TITGASGHVI

 81      DGNGQAYWDG KGSNSNSNQK

101      PDHFIVVQKT TGNSKITNLN

121      IQNWPVHCFD ITGSSQLTIS

141      GLILDNRAGD KPNAKSGSLP

161      AAHNTDGFDI SSSDHVTLDN

181      NHVYNQDDCV AVTSGTNIVV

201      SNMYCSGGHG LSIGSVGGKS

221      DNVVDGVQFL SSQVVNSQNG

241      CRIKSNSGAT GTINNVTYQN

261      IALTNISTYG VDVQQDYLNG

281      GPTGKPTNGV KISNIKFIKV

301      TGTVASSAQD WFILCGDGSC

321      SGFTFSGNAI TGGGKTSSCN

341      YPTNTCPS*
```

-continued

SEQUENCES

NUCLEOTIDE SEQUENCE of *Phaseolus vulgaris* PGIP2 (SEQ ID NO: 3)

ATGACTCAATTCAATATCCCAGTAACCATGTCTTCAAGCTTAAGCATAA

TTTTGGTCATTCTTGTATCTTTGAGCACTGCACACTCAGAGCTATGCAA

CCCACAAGACAAGCAAGCCCTTCTCCAAATCAAGAAAGACCTTGGCAAC

CCAACCACTCTCTCCTCATGGCTTCCAACCACCGACTGTTGCAACAGAA

CCTGGCTAGGTGTTTTATGCGACACCGACACCCAAACATATCGCGTCAA

CAACCTCGACCTCTCCGGCCTTAACCTCCCAAAACCCTACCCTATCCCT

TCCTCCCTCGCCAACCTCCCCTACCTCAATTTTCTATACATTGGTGGCA

TCAATAACCTCGTCGGTCCAATCCCCCCCGCCATCGCTAAACTCACCCA

ACTCCACTATCTCTATATCACCCACACCAATGTCTCCGGCGCAATACCC

GATTTCTTGTCACAGATCAAAACCCTCGTCACCCTCGACTTCTCCTACA

ACGCCCTCTCCGGCACCCTACCTCCCTCCATCTCTTCTCTCCCCAACCT

CGTCGGAATCACATTCGACGGCAACCGAATCTCCGGCGCCATCCCCGAC

TCCTACGGCTCATTTTCGAAGCTGTTCACGTCGATGACCATCTCCCGCA

ACCGCCTCACCGGGAAGATTCCGCCGACGTTTGCGAATCTGAACCTGGC

GTTCGTTGACTTGTCTCGAAACATGCTGGAGGGTGACGCGTCGGTGTTG

TTCGGATCAGATAAGAACACGCAGAAGATACATCTGGCGAAGAACTCTC

TTGCCTTTGATTTGGGGAAAGTGGGGTTGTCAAAGAACTTGAACGGGTT

GGATCTGAGGAACAACCGTATCTATGGGACGCTACCGCAGGGACTGACG

CAGCTAAAGTTTCTGCACAGTTTAAATGTGAGCTTCAACAATCTGTGCG

GTGAGATTCCTCAAGGTGGGAACTTGCAAAGATTTGACGTTTCTGCTTA

TGCCAACAACAAGTGCTTGTGTGGTTCTCCTCTTCCTGCCTGCACT

AMINO ACID SEQUENCE of *Phaseolus vulgaris* PGIP2 (SEQ ID NO: 4, Accession No. NCBI UNIPROT P58822.1)

ORIGIN

```
  1      MTQFNIPVTM SSSLSIILVI

 21      LVSLSTARSE LCNPQDKQAL

 41      LQIKKDLGNP TTLSSWLPTT

 61      DCCNRTWLGV LCDTDTQTYR

 81      VNNLDLSGLN LPKPYPIPSS

101      LANLPYLNFL YIGGINNLVG

121      PIPPAIAKLT QIHYLYITHT

141      NVSGAIPDFL SQIKTLVTLD

161      FSYNALSGTL PPSISSLPNL

181      VGITFDGNRI SGAIPDSYGS

201      FSKLFTSMTI SRNRLTGKIP

221      PTFANLNLAF VDLSRNMLEG

241      DASVLFGSDK NTQKIHLAKN

261      SLAFDLGKVG LSKNINGLDL

281      RNNRIYGTLP QGLTQLKFLH
```

-continued

SEQUENCES

301 *SLNVSFNNLC GEIPQGGNLQ*

321 *RFDVSAYANN KCLCGSPLPA*

341 *CT**

NUCLEOTIDE SEQUENCE OF THE OGM EXPRESSED IN PLANTS

(SEQ ID NO: 5)

ATGACTCAATTCAATATCCCAGTAACCATGTCTTCAAGCTTAAGCATAA

TTTTGGTCATTCTTGTATCTTTGAGCACTGCACACTCAGAGCTATGCAA

CCCACAAGACAAGCAAGCCCTTCTCCAAATCAAGAAAGACCTTGGCAAC

CCAACCACTCTCTCCTCATGGCTTCCAACCACCGACTGTTGCAACAGAA

CCTGGCTAGGTGTTTTATGCGACACCGACACCCAAACATATCGCGTCAA

CAACCTCGACCTCTCCGGCCTTAACCTCCCAAAACCCTACCCTATCCCT

TCCTCCCTCGCCAACCTCCCCTACCTCAATTTTCTATACATTGGTGGCA

TCAATAACCTCGTCGGTCCAATCCCCCCCGCCATCGCTAAACTCACCCA

ACTCCACTATCTCTATATCACCCACACCAATGTCTCCGGCGCAATACCC

GATTTCTTGTCACAGATCAAAACCCTCGTCACCCTCGACTTCTCCTACA

ACGCCCTCTCCGGCACCCTACCTCCCTCCATCTCTTCTCTCCCCAACCT

CGTCGGAATCACATTCGACGGCAACCGAATCTCCGGCGCCATCCCCGAC

TCCTACGGCTCATTTTCGAAGCTGTTCACGTCGATGACCATCTCCCGCA

ACCGCCTCACCGGGAAGATTCCGCCGACGTTTGCGAATCTGAACCTGGC

GTTCGTTGACTTGTCTCGAAACATGCTGGAGGGTGACGCGTCGGTGTTG

TTCGGATCAGATAAGAACACGCAGAAGATACATCTGGCGAAGAACTCTC

TTGCCTTTGATTTGGGGAAAGTGGGGTTGTCAAAGAACTTGAACGGGTT

GGATCTGAGGAACAACCGTATCTATGGGACGCTACCGCAGGGACTGACG

CAGCTAAAGTTTCTGCACAGTTTAAATGTGAGCTTCAACAATCTGTGCG

GTGAGATTCCTCAAGGTGGGAACTTGCAAAGATTTGACGTTTCTGCTTA

TGCCAACAACAAGTGCTTGTGTGGTTCTCCTCTTCCTGCCTGCACT

GCGGCCGCA*CCCTGCTCCGTGACTGAGTACTCTGGCCTCGCCACCGCTG*

TCTCATCCTGCAAAAACATCGTGCTCAACGGTTTCCAAGTCCCGACAGG

CAAGCAACTCGACCTATCCAGCCTCCAGAATGACTCGACCGTTACCTTC

AAGGGCACGACCACTTTTGCCACCACTGCTGATAACGACTTTAATCCTA

TCGTCATTAGTGGAAGTAACATCACTATCACTGGTGCATCTGGCCATGT

CATTGATGGCAACGGTCAGGCGTACTGGGATGGCAAAGGTTCTAACAGC

AATAGCAACCAAAAGCCCGATCACTTCATCGTTGTTCAGAAGACCACCG

GCAACTCAAAGATCACAAACCTAAATATCCAGAACTGGCCCGTTCACTG

CTTCGACATTACAGGCAGCTCGCAATTGACCATCTCAGGGCTTATTCTT

GATAACAGAGCTGGCGACAAGCCTAACGCCAAGAGCGGTAGCTTGCCCG

CTGCGCATAACACCGACGGTTTCGACATCTCGTCCAGTGACCACGTTAC

GCTGGATAACAATCATGTTTATAACCAAGATGATTGTGTTGCTGTTACT

TCCGGTACAAACATCGTCGTTTCTAACATGTATTGCTCCGGCGGCCATG

-continued

SEQUENCES

GTCTTAGTATCGGATCTGTTGGTGGAAAGAGCGACAATGTCGTTGATGG

TGTTCAGTTCTTGAGCTCGCAGGTTGTGAACAGTCAGAATGGATGTCGC

ATCAAGTCCAACTCTGGCGCAACTGGCACGATCAACAACGTCACCTACC

AGAACATTGCTCTCACCAACATCAGCACGTACGGTGTCGATGTTCAGCA

GGACTATCTCAACGGCGGCCCTACTGGAAAGCCGACCAACGGAGTCAAG

ATCAGCAACATCAAGTTCATCAAGGTCACTGGCACTGTGGCTAGCTCTG

CCCAGGATTGGTTTATTCTGTGTGGTGATGGTAGCTGCTCTGGATTTAC

CTTCTCTGGAAACGCTATTACTGGTGGTGGCAAGACTAGCAGCTGCAAC

TATCCTACCAACACTTGCCCCAGCTAG

Legend:
underlined: SEQUENCE CODING FOR PGIP2
italics: SEQUENCE CODING FOR FpPG
bold: sequence coding for the spacer composed of 3 alanines

AMINO ACID SEQUENCE OF OGM EXPRESSED IN PLANTS (inducible both by b-estradiol and under the control of the promoter PR-1)

(SEQ ID NO: 6)

ORIGIN

1 MTQFNIPVTM SSSLSIILVI

21 LVSLSTAHSE LCNPQDKQAL

41 LQIKKDLGNP TTLSSWLPTT

61 DCCNRTWLGV LCDTDTQTYR

81 VNNLDLSGLN LPKPYPIPSS

101 LANLPYLNFL YIGGINNLVG

121 PIPPAIAKLT QLHYLYITHT

141 NVSGAIPDFL SQIKTLVTLD

161 FSYNALSGTL PPSISSLPNL

181 VGITFDGNRI SGAIPDSYGS

201 FSKLFTSMTI SRNRLTGKIP

221 PTFANLNLAF VDLSRNMLEG

241 DASVLFGSDK NTQKIHLAKN

261 SLAFDLGKVG LSKNLNGLDL

281 RNNRIYGTLP QGLTQLKFLH

301 SLNVSFNNLC GEIPQGGNLQ

321 RFDVSAYANN KCLCGSPLPA

341 CT**AAA***PCSVT EYSGLATAVS*

361 *SCKNIVLNGF QVPTGKQLDL*

381 *SSLQNDSTVT FKGTTTFATT*

401 *ADNDFNPIVI SGSNITITGA*

421 *SGHVIDGNGQ AYWDGKGSNS*

441 *NSNQKPDHFI VVQKTTGNSK*

461 *ITNLNIQNWP VHCFDITGSS*

481 *QLTISGLILD NRAGDKPNAK*

-continued

SEQUENCES	
501	*SGSLPAAHNT DGFDISSSDH*
521	*VTLDNNHVYN QDDCVAVTSG*
541	*TNIVVSNMYC SGGHGLSIGS*
561	*VGGKSDNVVD GVQFLSSQVV*
581	*NSQNGCRIKS NSGATGTINN*
601	*VTYQNIALTN ISTYGVDVQQ*
621	*DYLNGGPTGK PTNGVKISNI*
641	*KFIKVTGTVA SSAQDWFILC*
661	*GDGSCSGFTF SGNAITGGGK*
681	*TSSCNYPTNT CPS**

Legend:
underlined: AMINO ACID SEQUENCE OF PGIP2
italics: AMINO ACID SEQUENCE OF FpPG
bold: AMINO ACID SEQUENCE FOR THE SPACER COMPOSED OF 3 ALANINES

NUCLEOTIDE SEQUENCE OF OGM EXPRESSED IN PICHIA (SEQ ID NO: 7)

GAGCTATGCAACCCACAAGACAAGCAAGCCCTTCTCCAAATCAAGAAAG

ACCTTGGCAACCCAACCACTCTCTCCTCATGGCTTCCAACCACCGACTG

TTGCAACAGAACCTGGCTAGGTGTTTTATGCGACACCGACACCCAAACA

TATCGCGTCAACAACCTCGACCTCTCCGGCCTTAACCTCCCAAAACCCT

ACCCTATCCCTTCCTCCCTCGCCAACCTCCCCTACCTCAATTTTCTATA

CATTGGTGGCATCAATAACCTCGTCGGTCCAATCCCCCCCGCCATCGCT

AAACTCACCCAACTCCACTATCTCTATATCACCCACACCAATGTCTCCG

GCGCAATACCCGATTTCTTGTCACAGATCAAAACCCTCGTCACCCTCGA

CTTCTCCTACAACGCCCTCTCCGGCACCCTACCTCCCTCCATCTCTTCT

CTCCCCAACCTCGTCGGAATCACATTCGACGGCAACCGAATCTCCGGCG

CCATCCCCGACTCCTACGGCTCATTTTCGAAGCTGTTCACGTCGATGAC

CATCTCCCGCAACCGCCTCACCGGGAAGATTCCGCCGACGTTTGCGAAT

CTGAACCTGGCGTTCGTTGACTTGTCTCGAAACATGCTGGAGGGTGACG

CGTCGGTGTTGTTCGGATCAGATAAGAACACGCAGAAGATACATCTGGC

GAAGAACTCTCTTGCCTTTGATTTGGGGAAAGTGGGGTTGTCAAAGAAC

TTGAACGGGTTGGATCTGAGGAACAACCGTATCTATGGGACGCTACCGC

AGGGACTGACGCAGCTAAAGTTTCTGCACAGTTTAAATGTGAGCTTCAA

CAATCTGTGCGGTGAGATTCCTCAAGGTGGGAACTTGCAAAGATTTGAC

GTTTCTGCTTATGCCAACAACAAGTGCTTGTGTGGTTCTCCTCTTCCTG

*CCTGCACT**GCGGCCGCA**CCCTGCTCCGTGACTGAGTACTCTGGCCTCGC*

CACCGCTGTCTCATCCTGCAAAAACATCGTGCTCAACGGTTTCCAAGTC

CCGACAGGCAAGCAACTCGACCTATCCAGCCTCCAGAATGACTCGACCG

TTACCTTCAAGGGCACGACCACTTTTGCCACCACTGCTGATAACGACTT

TAATCCTATCGTCATTAGTGGAAGTAACATCACTATCACTGGTGCATCT

GGCCATGTCATTGATGGCAACGGTCAGGCGTACTGGGATGGCAAAGGTT

-continued

SEQUENCES	
	CTAACAGCAATAGCAACCAAAAGCCCGATCACTTCATCGTTGTTCAGAA
	GACCACCGGCAACTCAAAGATCACAAACCTAAATATCCAGAACTGGCCC
	GTTCACTGCTTCGACATTACAGGCAGCTCGCAATTGACCATCTCAGGGC
	TTATTCTTGATAACAGAGCTGGCGACAAGCCTAACGCCAAGAGCGGTAG
	CTTGCCCGCTGCGCATAACACCGACGGTTTCGACATCTCGTCCAGTGAC
	CACGTTACGCTGGATAACAATCATGTTTATAACCAAGATGATTGTGTTG
	CTGTTACTTCCGGTACAAACATCGTCGTTTCTAACATGTATTGCTCCGG
	CGGCCATGGTCTTAGTATCGGATCTGTTGGTGGAAAGAGCGACAATGTC
	GTTGATGGTGTTCAGTTCTTGAGCTCGCAGGTTGTGAACAGTCAGAATG
	GATGTCGCATCAAGTCCAACTCTGGCGCAACTGGCACGATCAACAACGT
	CACCTACCAGAACATTGCTCTCACCAACATCAGCACGTACGGTGTCGAT
	GTTCAGCAGGACTATCTCAACGGCGGCCCTACTGGAAAGCCGACCAACG
	GAGTCAAGATCAGCAACATCAAGTTCATCAAGGTCACTGGCACTGTGGC
	TAGCTCTGCCCAGGATTGGTTTATTCTGTGTGGTGATGGTAGCTGCTCT
	GGATTTACCTTCTCTGGAAACGCTATTACTGGTGGTGGCAAGACTAGCA
	GCTGCAACTATCCTACCAACACTTGCCCCAGCTAG

Legend:
underlined: SEQUENCE CODING FOR PGIP2 (corresponding to the region nt. 88-1026 of SEQ ID NO: 3)
italics: SEQUENCE CODING FOR FpPG
bold: sequence coding for the spacer composed of 3 alanines

AMINO ACID SEQUENCE OF OGM EXPRESSED IN PICHIA (SEQ ID NO: 8)

ORIGIN

1	ELCNPQDKQA LLQIKKDLGN
21	PTTLSSWLPT TDCCNRTWLG
41	VLCDTDTQTY RVNNLDLSGL
61	NLPKPYPIPS SLANLPYLNF
81	LYIGGINNLV GPIPPAIAKL
101	TQLHYLYITH TNVSGAIPDF
121	LSQIKTLVTL DFSYNALSGT
141	LPPSISSLPN LVGITFDGNR
161	ISGAIPDSYG SFSKLFTSMT
181	ISRNRLTGKI PPTFANLNLA
201	FVDLSRNMLE GDASVLFGSD
221	KNTQKIHLAK NSLAFDLGKV
241	GLSKNLNGLD LRNNRIYGTL
261	PQGLTQLKFL HSLNVSFNNL
281	CGEIPQGGNL QRFDVSAYAN
301	NKCLCGSPLP ACT**AAA***PCSV*
321	*TEYSGLATAV SSCKNIVLNG*
341	*FQVPTGKQLD LSSLQNDSTV*

-continued

	SEQUENCES
361	*TFKGTTTFAT TADNDFNPIV*
381	*ISGSNITITG ASGHVIDGNG*
401	*QAYWDGKGSN SNSNQKPDHF*
421	*IVVQKTTGNS KITNLNIQNW*
441	*PVHCFDITGS SQLTISGLIL*
461	*DNRAGDKPNA KSGSLPAAHN*
481	*TDGFDISSSD HVTLDNNHVY*
501	*NQDDCVAVTS GTNIVVSNMY*
521	*CSGGHGLSIG SVGGKSDNVV*
541	*DGVQFLSSQV VNSQNGCRIK*
561	*SNSGATGTIN NVTYQNIALT*
581	*NISTYGVDVQ QDYLNGGPTG*
601	*KPTNGVKISN IKFIKVTGTV*
621	*ASSAQDWFIL CGDGSCSGFT*
641	*FSGNAITGGG KTSSCNYPTN*
661	*TCPS**

Legend:
underlined: PROTEIN SEQUENCE OF PGIP2 (corresponding to the region aa. 30-342 of the SEQ ID NO: 4)
italics: PROTEIN SEQUENCE OF FpPG
bold: protein sequence coding of the spacer composed of 3 alanines

NUCLEOTIDE SEQUENCE OF OGM FUSED TO PROMOTER PR-1 for the expression of the chimeric protein after a pathogen attack

(SEQ ID NO 9)

<u>AAGCTTGTTTTAACTTATAAAATGATTCTCCCTCCATATAAAAAAGTTT</u>

<u>GATTTTATAGAATGTTTATACCGATTAAAAAAATAATAATGCTTAGTTA</u>

<u>TAAATTACTATTTATTCATGCTAAACTATTTCTCGTAACTATTAACCAA</u>

<u>TAGTAATTCATCAAATTTTAAAATTCTCAATTAATTGATTCTTGAAATT</u>

<u>CATAACCTTTTAATATTGATTGATAAAAATATACATAAACTCAATCTTT</u>

<u>TTAATACAAAAAAACTTTAAAAAATCAATTTTTCTGATTCGGAGGGAGT</u>

<u>ATATGTTATTGCTTAGAATCACAGATTCATATCAGGATTGGAAAATTTT</u>

<u>AAAGCCAGTGCATATCAGTAGTCAAAATTGGTAAATGATATACGAAGGC</u>

<u>GGTACAAAATTAGGTATACTGAAGATAGAAGAACACAAAAGTAGATCGG</u>

<u>TCACCTAGAGTTTTTCAATTTAAACTGCGTATTAGTGTTTGGAAAAAAA</u>

<u>AAACAAAGTGTATACAATGTCAATCGGTGATCTTTTTTTTTTTTTTTTT</u>

<u>TTTTTTTTTTCTTTTTGGATAAATCTCAATGGGTGATCTATTGACTGTT</u>

<u>TCTCTACGTCACTATTTTACTTACGTCATAGATGTGGCGGCATATATTC</u>

<u>TTCAGGACTTTTCAGCCATAGGCAAGAGTGATAGAGATACTCATATGCA</u>

<u>TGAAACACTAAGAAACAAATAATTCTTGACTTTTTTTCTTTTATTTGAA</u>

<u>AATTGACTGTAGATATAAACTTTTATTTTTTCTGACTGTAAATATAATC</u>

<u>TTAATTGCCAAACTGTCCGATACGATTTTTCTGTATTATTTACAGGAAG</u>

-continued

	SEQUENCES
	<u>ATATCTTCAAAACATTTTGAATGAAGTAATATATGAAATTCAAATTTGA</u>
	<u>AATAGAAGACTTAAATTAGAATCATGAAGAAAAAAAAAAACACAAAACAA</u>
	<u>CTGAATGACATGAAACAACTATATACAATGTTTCTTAATAAACTTCATT</u>
	<u>TAGGGTATACTTACATATATACTAAAAAAATATATCAACAATGGCAAAG</u>
	<u>CTACCGATACGAAACAATATTAGGAAAAATGTGTGTAAGGACAAGATTG</u>
	<u>ACAAAAAAATAGTTACGAAAACAACTTCTATTCATTTGGACAATTGCAA</u>
	<u>TGAATATTACTAAAATACTCACACATGGACCATGTATTTACAAAAACGT</u>
	<u>GAGATCTATAGTTAACAAAAAAAAAAAGAAAAAAATAGTTTTCAAATCT</u>
	<u>CTATATAAGCGATGTTTACGAACCCCAAAATCATAACACAACAATAACC</u>
	<u>ATTATCAACTTAGAAAA</u>*ATGACTCAATTCAATATCCCAGTAACCATGTC*
	TTCAAGCTTAAGCATAATTTTGGTCATTCTTGTATCTTTGAGCACTGCA
	CACTCAGAGCTATGCAACCCACAAGACAAGCAAGCCCTTCTCCAAATCA
	AGAAAGACCTTGGCAACCCAACCACTCTCTCCTCATGGCTTCCAACCAC
	CGACTGTTGCAACAGAACCTGGCTAGGTGTTTTATGCGACACCGACACC
	CAAACATATCGCGTCAACAACCTCGACCTCTCCGGCCTTAACCTCCCAA
	AACCCTACCCTATCCCTTCCTCCCTCGCCAACCTCCCCTACCTCAATTT
	TCTATACATTGGTGGCATCAATAACCTCGTCGGTCCAATCCCCCCCGCC
	ATCGCTAAACTCACCCAACTCCACTATCTCTATATCACCCACACCAATG
	TCTCCGGCGCAATACCCGATTTCTTGTCACAGATCAAAACCCTCGTCAC
	CCTCGACTTCTCCTACAACGCCCTCTCCGGCACCCTACCTCCCTCCATC
	TCTTCTCTCCCCAACCTCGTCGGAATCACATTCGACGGCAACCGAATCT
	CCGGCGCCATCCCCGACTCCTACGGCTCATTTTCGAAGCTGTTCACGTC
	GATGACCATCTCCCGCAACCGCCTCACCGGGAAGATTCCGCCGACGTTT
	GCGAATCTGAACCTGGCGTTCGTTGACTTGTCTCGAAACATGCTGGAGG
	GTGACGCGTCGGTGTTGTTCGGATCAGATAAGAACACGCAGAAGATACA
	TCTGGCGAAGAACTCTCTTGCCTTTGATTTGGGGAAAGTGGGGTTGTCA
	AAGAACTTGAACGGGTTGGATCTGAGGAACAACCGTATCTATGGGACGC
	TACCGCAGGGACTGACGCAGCTAAAGTTTCTGCACAGTTTAAATGTGAG
	CTTCAACAATCTGTGCGGTGAGATTCCTCAAGGTGGGAACTTGCAAAGA
	TTTGACGTTTCTGCTTATGCCAACAACAAGTGCTTGTGTGGTTCTCCTC
	*TTCCTGCCTGCACT***GCGGCGCGA***CCCTGCTCCGTGACTGAGTACTCTG*
	GCCTCGCCACCGCTGTCTCATCCTGCAAAAACATCGTGCTCAACGGTTT
	CCAAGTCCCGACAGGCAAGCAACTCGACCTATCCAGCCTCCAGAATGAC
	TCGACCGTTACCTTCAAGGGCACGACCACTTTTGCCACCACTGCTGATA
	ACGACTTTAATCCTATCGTCATTAGTGGAAGTAACATCACTATCACTGG
	TGCATCTGGCCATGTCATTGATGGCAACGGTCAGGCGTACTGGGATGGC
	AAAGGTTCTAACAGCAATAGCAACCAAAAGCCCGATCACTTCATCGTTG
	TTCAGAAGACCACCGGCAACTCAAAGATCACAAACCTAAATATCCAGAA
	CTGGCCCGTTCACTGCTTCGACATTACAGGCAGCTCGCAATTGACCATC

-continued

SEQUENCES
TCAGGGCTTATTCTTGATAACAGAGCTGGCGACAAGCCTAACGCCAAGA
GCGGTAGCTTGCCCGCTGCGCATAACACCGACGGTTTCGACATCTCGTC
CAGTGACCACGTTACGCTGGATAACAATCATGTTTATAACCAAGATGAT
TGTGTTGCTGTTACTTCCGGTACAAACATCGTCGTTTCTAACATGTATT
GCTCCGGCGGCCATGGTCTTAGTATCGGATCTGTTGGTGGAAAGAGCGA
CAATGTCGTTGATGGTGTTCAGTTCTTGAGCTCGCAGGTTGTGAACAGT
CAGAATGGATGTCGCATCAAGTCCAACTCTGGCGCAACTGGCACGATCA
ACAACGTCACCTACCAGAACATTGCTCTCACCAACATCAGCACGTACGG
TGTCGATGTTCAGCAGGACTATCTCAACGGCGGCCCTACTGGAAAGCCG
ACCAACGGAGTCAAGATCAGCAACATCAAGTTCATCAAGGTCACTGGCA
CTGTGGCTAGCTCTGCCCAGGATTGGTTTATTCTGTGTGGTGATGGTAG
CTGCTCTGGATTTACCTTCTCTGGAAACGCTATTACTGGTGGTGGCAAG
ACTAGCAGCTGCAACTATCCTACCAACACTTGCCCCAGCTAG
Legend:
underlined: SEQUENCE OF THE PROMOTER PR-1
italics: SEQUENCE CODING FOR THE OGM
bold: SEQUENCE CODING FOR THE LINKER OF 3 ALANINES

The present invention will be illustrated with non-limiting examples in reference to the following figures.

FIGS. **1**A-**1**G (collectively referred to as FIG. **1**). Characterisation of the transgenic plants expressing OGM inducible by chemical treatment. (A) Schematic representation of two OGM molecules that interact. PvPGIP2 and FpPG are linked by three alanines and correspond to the N and C terminals of the fusion protein, respectively. (B) Four-week-old plants of a representative transgenic line which express the OGM after one week of induction with β-estradiol. (C) The total protein extracts from the leaves of the rosette (3 μng) of 4-week-old plants of a representative transgenic line which express the OGM after induction with β-estradiol at the times indicated were separated by SDS-PAGE and analysed by means of an immunodecoration assay using the antibody directed against FpPG as the primary one. The purified OGM (+, 80 kDa) and FpPG (PG, 37 kDa) were used as reference proteins. (D) Determination of polygalacturone activity in the protein extracts (3 μg) obtained from leaves of transgenic plants treated, for the times indicated, with DMSO (−) or β-estradiol (+) by means of an agar diffusion assay. (E) Visualisation of callose deposits in the leaves of the rosette of the transgenic plants treated with DMSO (non-induced) or β-estradiol for 170 h by staining with aniline blue. (F) The expression of the genes WRKY40 and RetOx was determined in the transgenic plants treated with β-estradiol for the times indicated by semi-quantitative RT-PCR, using the expression of the gene UBQ5 as reference. (G) Wild plants (WT) and transgenic plants were treated for 170 h with β-estradiol and subsequently inoculated with a suspension of spores of *B. cinerea*. After two days, the area of the lesion generated by the fungus was measured. The bars indicate the mean lesion area produced by the fungus (n>10). The asterisk indicates a statistically significant difference, in accordance with the Student t-test (P<0.05). This experiment was repeated three times with comparable results.

FIGS. **2**A-**2**F (collectively referred to as FIG. **2**). Inducible release of oligogalacturonides from plants expressing the OGM. (A-E) HPAEC-PAD analysis on pectin-enriched fractions of cell walls extracted from plants belonging to a representative transgenic line expressing the OGM under the control of a β-estradiol-inducible promoter. Four-week-old plants were treated with the inducer for 0 (A), 24 (B), 70 (C) and 170 hours (D) prior to extraction. A preparation of OGs purified with a degree of polymerisation (DP) of between 6 and 16 was used as a reference (E). The chromatograms show the signal intensity (nC, y axis) as a function of the retention time (minutes, X axis). (F) MALDI-TOF analysis of the same pectin fraction indicated in (D). The numbers indicate the DP of the oligogalacturonides identified as sodium adducts of the same mass as the corresponding peak. The graph shows the intensity of the signals (expressed as a percentage, Y axis) as a function of the mass of the ion (m/z, X axis).

FIGS. **3**A-**3**F (collectively referred to as FIG. **3**). Pathogen-inducible expression of the OGM imparts an increased resistance to microbial infections. (A) Four-week-old plants belonging to two independent lines expressing the OGM under the control of the pathogen-inducible promoter which regulates the expression of PR1 (P_{PR1}::OGM 1 and 2) were inoculated with a suspension of spores of *B. cinerea* and the levels of expression of the transgene were quantified before (grey bars) and two days after infection (black bars) by quantitative PCR, using the expression of the gene UBQ5 as reference. The bars indicate the mean level of expression (in arbitrary units)±SD (n=3). (B) The accumulation of the OGM in leaves of wild type plants (WT) and transgenic plants before (−) and two days after inoculation with *B. cinerea* (+). The total protein extract (30 μg) was separated by SDS-PAGE and subjected to an immunodecoration assay, using a primary antibody directed against FpPG (C-D) The leaves of the rosette of wild type plants (WT), P_{PR1}::OGM line 1 and line 2 were inoculated with *B. cinerea* and—after 72 h—a determination was made both of the percentage of infections that took hold, measured as a percentage of inocula that developed grey rot lesions (C) and the mean area of the lesions (D). The bars indicate (C) the mean of three independent experiments (n>12 in each experiment); the bars in (D) indicate the mean area±SE (n>12). (E) The leaves of the rosette of wild type plants (WT), P_{PR1}::OGM line 1 and line 2 were inoculated with *P. carototovorum* and the area of the lesions was measured after 16 hours. The bars indicate the mean area of the lesions±SE (n>12). (f) The leaves of the rosette of untransformed plants (WT), P_{PR1}::OGM line 1 and line 2 were inoculated with *P. syringae* pv tomato DC3000 and the bacterial growth within the inoculated tissue was determined at the times indicated. The bars indicate the colony-forming units (cfu) per cm^2 of leaf tissue (n=6). The asterisks in (D-F) indicate the statistically significant differences between the control plant and the transgenic plants, in accordance with Fischer's exact test (C) or the Student t-test (D-F). *, P<0.05; ***, P<0.01. The experiments in (D-F) were repeated three times with comparable results.

The transgenic plants belonging to both lines were significantly more resistant to infection, showing a 75% reduction in the bacterial load detected in the tissues compared to that observed in wild type plants (WT).

FIGS. **4**A-**4**C (collectively referred to as FIG. **4**). Biochemical characterisation of the OGM expressed in *P. pastoris*. (A) SDS-PAGE analysis (7.5% acrylamide) on the purified fusion protein (OGM) and the one subjected to crosslinking (OGM-cl), where it is possible to detect the

formation of multimers ranging from the dimer to the tetramer and the disappearance of the monomer. (B) Top panel, evaluation by agar diffusion assay of the polygalacturone activity carried out by 220 ng of purified OGM and by 1 ng of purified FpPG; bottom panel, immunodecoration analysis of the same protein samples using an antibody directed against the FpPG The expected molecular weights of the OGM (80 kDa) and FpPG (35 kDa) are shown. In the culture filtrate of the untransformed (mock) *P. pastoris* neither the activity nor the presence of the protein were detected. (C) The fractions eluted by affinity chromatography AnPGII were subjected to SDS-PAGE analysis (10% acrylamide) and visualised by Ag staining. The OGM (80 KDa) was eluted in the fractions. Ft represents the fraction containing the proteins not bound to the column.

FIG. **5**. The OGs released by plants expressing the OGM are hydrolysed by polygalacturonase. A fraction of the cell wall of leaves of the rosette enriched in pectin was extracted from the transgenic plant expressing the OGM under the control of the β-estradiol-inducible promoter 170 hours after the time of induction. The pectic fractions were analysed by HPAEC-PAD before (−) and after treatment with 5 μg of pure FpPG (+). The chromatogram shows the intensity of the signals (nC, Y axis) as a function of the retention times (minutes, X axis).

EXAMPLE

Materials and Methods

Strains

E. coli DH5α [genotype: F-φ80lacZΔM15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hsdR17(rk, mk+) phoA supE44 thi-1 gyrA96 relA1 λ-(Invitrogen)

A. tumefaciens LBA4404 (INVITROGEN, catalogue number: 18313-015)

P. pastoris X33 (wild type) (Invitrogen)

A. thaliana Col-0 (wild type) (purchased from Lehle Seeds)

Construction of the Gene Cassette for the Expression of the Chimeric Fusion Protein PG-PGIP (OGM) in *P. pastoris* (Corresponding to the Amino Acid Sequence of the OGM Expressed in *Pichia* (SEQ ID NO:8))

For the expression of the fusion protein in *P. pastoris*, the 5' end of the gene coding for PvPGIP2 was fused to the sequence coding for the alpha factor of yeast present in the integrative vector pGAPZ alpha which enables the constitutive expression of the transgene; the construct pGAPZα-PGIP2 was thus obtained. The gene coding for the mature protein PvPGIP2 was amplified by means of specific primers (EcoRIPGIP2Fw (SEQ ID NO: 10) and NotIPGIP2Rv (SEQ ID NO: 11)) which introduced the "EcoRI" and "NotI" restriction sites at the 5' and 3' ends, respectively; the gene was then cloned at the multiple cloning site of the vector by exploiting the aforesaid restriction sites. In parallel, "NotI" and "XbaI" restriction sites were introduced at the 5' and 3' ends, respectively, of the sequence coding for FpPG using specific primers (NotIFpPGFw (SEQ ID NO: 12) and XbaIFpPGRv (SEQ ID NO: 13)) via PCR. The primer which readapted the SI end of the FpPG gene by inserting the restriction site NotI maintained the correct reading frame between the PvPGIP2 and FpPG and introduced 9 further nucleotide bases, which would code the peptide linker composed of 3 alanines. The fragment coding for FpPG was thus cloned in the multiple cloning site using the "NotI" and "XbaI" restriction sites. The gene fusion (called OG-machine; abbreviated as OGM) was sequenced to exclude the presence of undesirable mutations. The recombinant plasmid was linearised by means of the AvrII restriction enzyme, necessary for site-specific recombination in *P. pastoris*. The transformation, selection and growth of *Pichia* took place according to the instructions given in the Invitrogen manual. The filtrates of cultures grown for 3 days were tested both by means of an agar diffusion assay and an immunodecoration assay. The OGM was purified using the same procedure as used to purify PvPGIP2 from a culture filtrate of *P. pastoris* as described in (29).

Primers used for the construction of gene cassettes coding the OGM for expression in *P. pastoris* and β-estradiol-inducible expression in *A. thaliana*:

EcoRIPGIP2Fw: (SEQ ID NO: 10)
5' ATCGATGAATTCGAGCTATGCAACCCACA 3'

NotIPGIP2Rv: (SEQ ID NO: 11)
5'-TCTTCTAAGTGCGGCCGCAGTGCAGGCAGGAAGAG-3'

NotIFpPGFw: (SEQ ID NO: 12)
5'-TCAACACTATGCGGCCGCACCCTGCTCCGTGACTGAG-3'

XbaIFpPGRv: (SEQ ID NO: 13)
5'-ATCGATTCTAGACTAGCTGGGGCAAGTGTTG-3'

AvrIISP1Fw: (SEQ ID NO: 14)
5'-ACTAAGCCTAGGACTATCTAGAATGACTCAATTCAATATCCCAG-3'

EheIPGIP2Rv: (SEQ ID NO: 15)
5'-GGGGATGGCGCCGGAG-3'

XhoISP1Fw: (SEQ ID NO: 16)
5'-ACTAAGCTCGAGATGACTCAATTCAATATCCCAG-3'

PacIFpPGRv: (SEQ ID NO: 17)
5'-CCTAAGTTAATTAACTAGCTGGGGCAAGTGTTG-3'

The underlined sequences indicate the restriction sites introduced.

Molecular Crosslinking Experiment Conducted on the OGM

1 μg of pure OGM was subjected to auto-crosslinking in a volume of 50 μL of a solution containing 50 mM sodium acetate pH 4.6, to which methanol-free formaldehyde was added at the final concentration of 1% (Thermo-Fisher Scientific, U.S.A). The reaction was incubated at a temperature of 28° C. for 16 hours. 2 μL of the reaction was analysed via SDS-PAGE.

Preparation of the Construct for β-Estradiol-Inducible Expression in *A. thaliana*

The cDNA coding for the signal peptide of PvPGIP2 (SP), available already fused to PvPGIP2 (27), was amplified up to the EheI restriction site located in the sequence of PvPGIP2 a+550 pairs of bases from the first ATG using the primers called AvrIISP1Fw and EheIPGIP2Rv (SEQ ID NO:14 and SEQ ID NO: 15, respectively). The amplified fragment was cloned in the construct used for expression in *P. pastoris* using the restriction sites of the AvrII and EheI enzymes. The new construct obtained, which consisted in a fusion between the cDNA coding for the OGM and the sequence coding for the signal peptide for the secretion of PvPGIP2 into the apoplast was thus introduced by means of electroporation in *E. coli* DH5α for amplification of the plasmid.

The cDNA coding for the fusion protein fused to the signal peptide for the secretion of PvPGIP2 was amplified by PCR using the specific XhoISP1Fw and PacIFpPGRv primers (SEQ ID NO:16 and SEQ ID NO: 17, respectively) which introduced the XhoI and PacI restriction sites at the 5^{I} and 3^{I} ends of the transgene, respectively. The gene readapted to the ends was then cloned in the vector pMDC7 for β-estradiol-inducible expression in plants (31) using the same restriction sites.

The final construct pMDC7.SP-OGM was amplified in *E. coli* DH5α, and then plasmid extraction took place; the purified plasmid was introduced in *A. tumefaciens* LBA4404 by electroporation.

Stable Transformation of *A. thaliana*

The transgenic plants of *Arabidopsis thaliana* ecotype Col-0 were generated by infection of the flower primordia mediated by *A. tumefaciens* according to the procedure described in (41). Following the transformation, the transgenic lines were selected in generation T1 by seeding on a plate containing MS solid medium and hygromycin (23 mg L-1) as a positive selection marker. The transgenic lines of the hygromycin-resistant T2 generation were selected for subsequent analyses; in particular, the lines of the T2 generation that showed a segregation of the transgene in a 3:1 ratio were isolated for selection of the transforming homozygote in the T3 generation.

Induction of Expression in the Transgenic Lines of the T3 Generation Using β-Estradiol

XVE is a transcriptional chimeric factor constitutionally expressed in the nucleus of the plant cell transformed with the T-DNA deriving from the vector pMDC7. XVE is capable of transcribing the transgene regulated by the inducible promoter OlexA-46 only in the presence of β-estradiol (31). The induction of expression was achieved by spraying 1.5 mL of a solution containing 50 μM β-estradiol per transgenic plant.

Analysis of Gene Expression by Semiquantitative RT-PCR

The removed leaf tissues were frozen in liquid nitrogen, homogenised by means of an MM301 Ball Mill (Retsch), and the total RNA was extracted using Isol-RNA Lysis Reagent (5 Prime), following the instructions provided in the manufacturer's manual. The RNA was treated with RQ1 DNase (PROMEGA) and the first strand of the cDNA was synthesized using the reverse transcript ImProm-II (PROMEGA), in accordance with the manufacturer's instructions. The expression levels of each gene relative to the expression of the gene UBQ5 were determined using a modification of the Pfaffl method (42) as previously described in (43). The (RT)-PCR reaction was conducted in a 50-μL reaction mixture containing 2 μL of cDNA, 1× buffer (RBCBioscience), 3 mm MgCl2, 100 μm of each dNTP, 0.5 μm of each primer (primers EcoRIPGIP2Fw (SEQ ID NO:10) and EheIPGIP2Rv (SEQ ID NO:15)) and 1 unit of Taq polymerase. 25, 30, and 35 amplification cycles were carried out by PCR for every pair of primers in order to verify the linearity of the amplification reaction. The PCR products were separated by agarose gel electrophoresis and visualised by means of ethidium bromide.

Immunodecoration Assay

The extraction of total proteins from leaf tissue was carried out using a buffer consisting of 20 mM sodium acetate pH 4.6 and 1M NaCl, in which the leaves previously homogenised by means of an MM301 Ball Mill (Retsch) were incubated for 20 minutes. The same quantities of total proteins were separated by SDS-PAGE analysis and then transferred onto a nitrocellulose membrane using, as the transfer buffer, a solution containing 25 Mm TRIS, 192 mM glycine, pH 8.3, and 20% methanol at the temperature of 4° C. for 1 h. Following the transfer, the filter was stained with Ponceau S in order to verify equal loading for all samples; then the filter was saturated by incubating it for 2 h in a solution consisting of 50 mM phosphate buffer, 150 mM NaCl and 3% bovine serum albumin (BSA, SIGMA ALDRICH); subsequently, the filter was incubated for 12 h with a primary antibody directed toward the FpPG (40). After suitable washing, the membrane was incubated with a secondary antibody conjugated to horseradish peroxidase (Amersham, UK) for approximately 2 h. The membrane was washed again and treated with ECL reagents (Amersham, UK) in order to promote the detection of the transgenic protein.

Determination of Callose Deposition

Leaves of 5-week-old plants were sprayed with a solution containing 50 μM β-estradiol. After 170 h, about 4 leaves were collected from 3 independents plants and dehydrated in a solution consisting of 100% ethanol for about 2 hours. The leaves were then incubated for 15 minutes in 75% ethanol and, finally, in 50% ethanol. Following this pretreatment the leaves were washed in 150 mM phosphate buffer pH 8.0 and then stained for 1 h at 25° C. in 150 mM phosphate buffer, pH 8.0, containing 0.01% (w/v) aniline blue. After staining, the leaves were incubated in 50% glycerol and examined by epifluorescence UV using an Axioskop 2 plus microscope (Zeiss). Pictures were taken with a ProgRes C10 3.3 Megapixel colour digital camera (Jenoptik).

Infection Assays

Botrytis cinerea was propagated in a solid medium consisting of 20 g l-1 malt extract, 10 g l-1 peptone (Difco, Detroit, USA), and 15 g l-1 agar for 7-10 days at +24° C. with a photoperiod of 12 h prior to collection of the spores. The rosette leaves of *Arabidopsis* plants were placed on Petri plates containing 0.8% agar, with the petiole inserted into the solid medium to act as a support. Inoculation was carried out by placing on each side of the central vein of each leaf 5 microliters of a solution consisting of PDB liquid medium (PDB; Difco, Detroit, USA) containing a suspension of 5×105 conidiospores mL-1. The plates were incubated at 22° C. under constant light for 2 days. A high level of humidity was maintained by covering the plates with transparent film. Under the experimental conditions, the majority of the infections produced a rapid expansion of rot lesions of comparable diameter. The size of the lesion was determined by measuring the diameter or, in the case of oval lesions, the major axis of the necrotic area.

P. carotovorum subsp. *carotovorum* (formerly *E. carotovora* subsp. *carotovora*) was obtained from DSMZ GmbH Germany (strain DSMZ 30169). Following growth in Luria-Bertani (LB) liquid medium, the bacteria were suspended in 50 mM potassium phosphate buffer (pH 7.0) and inoculated at a concentration of 5×10^{7} cells mL-1. In each experiment, 12 mature leaves were collected (3 leaves per plant) and placed on damp filter paper in Petri capsules; they were then inoculated with 5 microliters of the bacterial suspension and maintained at 22° C. and with a photoperiod of 12 hours. The area of the lesions was obtained by measuring the surface of the macerated tissue 16 hours after infection. The areas were measured with ImageJ software (WS Rasband, ImageJ; National Institutes of Health, Bethesda, Md., USA). The experiment was repeated three times with different lots of plants, and a statistical analysis of the results was conducted by unidirectional analysis of variance (ANOVA), followed by the Student-Tukey range test.

Pseudomonas syringae pv *tabaci* DC3000 was propagated in LB liquid medium at 28° C. for 1 day; the bacterial suspension was resuspended in 10 mM $MgCl_2$ (1×10^6 cell/ml). The inoculations were carried out by infiltrating the bacterial suspension using a 1 ml needleless syringe.

Isolation and Detection of Oligogalacturonides in the Transgenic Plants

Leaves (approximately 100 mg per sample) belonging to transgenic and wild type plants about 4 weeks old were frozen in liquid nitrogen following induction with beta-estradiol and homogenised using a Retschmill machine (model MM200; Retsch) at 25 Hz for 1 min. The pulverised tissue was washed twice using 1 mL of a solution consisting of 70% ethanol and precipitated by centrifugation at 20,000×g for 10 min. The precipitate was then washed twice with a chloroform:methanol mixture (1:1, v/v) and centrifuged at 20000×g for 10 minutes. After centrifugation, the precipitate was suspended twice with acetone and again precipitated by centrifugation at 20000×g for 10 min. The pellet obtained was incubated overnight under the air flow of a chemical fume hood in order to favour evaporation of the solvent, and then resuspended using 200 μL of Ch buffer (composition of the Ch buffer: 50 mM ammonium acetate pH 5, 50 mM CDTA and 50 mM ammonium oxalate) for two hours under stirring at room temperature. The supernatant was recovered after centrifugation at 20000×g for 10 minutes.

Analysis by High-Performance Anion Exchange Chromatography (HPAEC) Coupled to a Pulsed Amperometric Detector (PAD)

Analysis of the oligogalacturonides was carried out by HPAEC-PAD. The HPAEC system (ICS-3000, Dionex Corporation, Sunnyvale, Calif., USA) was equipped with a CarboPac PA-200 separation column (2 mm ID×250 mm; Dionex Corporation) and a Carbopac PA-200 guard column (2 mm ID×50 mm; Dionex Corporation). A flow of 0.4 mL min-1 was used at a constant temperature of 25° C. The samples, with an injection volume of 25 μL, were separated using a gradient consisting of 0.05 M KOH (A) and 1 M KOAc in 0.05 M KOH (B) according to the following elution program: 0-30 min from 20% B to 80% B, 30-32 min to 100% B. Prior to the injection of each sample, the column was balanced with 90% A and 10% B for 10 min.

Treatment of Pectin-Enriched Fractions by Means of Exogenous PG

The pectin-enriched fraction extracted from 20 mg of leaf tissue was treated with 5 μg of pure FpPG for 1 hour at a temperature of 37° C. Subsequently, the reaction mixture was subjected to HPAEC-PAD analysis.

Preparation of the Construct for the Expression of the OGM Under the Control of the Inducible Promoter PR-1 in *A. thaliana*

The primers for preparing the construct for the pathogen-inducible expression of the OGM under the control of the promoter that regulates the expression of the gene PR-1 (AT2G14610, Accession No. UNIPROT Q39187) in *A. thaliana* are the following (the underlined sequence indicates the restriction site introduced):

XbaISP1Fw:
A:
(SEQ ID NO: 18)
5'-GACTATCTAGAATGACTCAATTCAATATCCC-3';

HindIIIPR1Fw:
B:
(SEQ ID NO: 19)
5'-GTTAGCA CAAGCTTGTT TTAAC-3';

-continued

XbaIPacIFpPGRv:
C:
(SEQ ID NO: 20)
5'-CCTAAGTCTAGAGGTCTTAATTAACTAGCTGGGG-3';

HindIIIPGIP2Rv
D:
(SEQ ID NO: 21)
5'-TGCTTAAGCTTGAAGACATGGTTACTGGGATATTGAATTGAGTCA
TTTTTCTAAGTTGATAATGG-3'.

As the starting plasmid, the cloning procedure used the vector pBI121 (Chen P Y, Wang C K, Soong S C, To K Y: Complete sequence of the binary vector pBI121 and its application in cloning T-DNA insertion from transgenic plants. Mol Breeding 2003 11(4): 287-293), in which the PacI restriction site was introduced upstream (−6) of the SacI restriction site, located at the 3' end of the gene coding for beta-glucuronidase. The gene coding for beta-glucuronidase was then excised via the restriction sites XbaI and PacI. Subsequently, the OGM fused to the signal peptide of PGIP2 was amplified via the primers XbaISP1Fw (SEQ ID NO: 18) and XbaIPacIFpPGRv (SEQ ID NO: 20) which introduced XbaI and PacI at the 5' and 3' ends, respectively, of the transgene and cloned in the vector pBI121 from which the gene coding for beta-glucuronidase were previously removed. As a second step, the terminal portion of the promoter which regulates the expression of the gene PR-1 (1300 base pairs of AT2G14610), including the 5' UTR sequence was amplified from the gDNA of *A. thaliana* Col-0 using specific primers (HindIIIPR1Fw (SEQ ID NO: 19) and D (SEQ ID NO: 21)) which introduce the restriction sites HindIII at both ends of the amplified product. As a result, the primer of the antisense strand HindIIIPGIP2Rv readapted the 3' end of the amplicon, introducing a nucleotide tail consisting of the first 39 bases coding for the signal peptide of PGIP2, which is characterised by the HindIII restriction site in its native sequence. The final product obtained was a transcriptional fusion between the last 1300 pairs of bases of the promoter PR-1, the 5' UTR region of the gene PR-1 and the sequence of the signal peptide of PvPGIP2 coding for the first 13 amino acids downstream of the first methionine. The fragment was cloned using the restriction site HindIII of the plasmid pBI121 (containing the gene OGM), previously digested with HindIII and dephosphorylated by alkaline phosphatase. It is worth noting that the digestion of pBI121 via the enzyme HindIII provokes the excision of a DNA fragment of 900 pairs of bases corresponding to the promoter 35S. The cassette was sequenced both to exclude the presence of undesirable mutations and to verify the correct orientation of the truncated version of the promoter PR-1.

Results

In order to exploit the potentialities of OGs as generic plant elicitors during defence responses, we engineered a chimeric fusion protein comprising PvPGIP2 (Uniprot Accession Number: P58822.1; SEQ ID NO:4), an inhibitor originating from the common pea (*Phaseolus vulgaris*) (27,28), and a PG ligand (FpPG) thereof, originating from the fungus *Fusarium phyllophilum* (SEQ ID NO:2; corresponding to aa.26-aa.373 of the Uniprot sequence Accession Number: Q07181.1)(29). In this manner, the enzyme and its inhibitor will be simultaneously expressed in a stoichiometric ratio of 1:1, which results in an increase, in vivo, in the production of biologically active OGs. In *Pichia pastoris* and *Arabidopsis thaliana*, fusion proteins were expressed with linkers consisting of the module Gly4Ser1 repeated from seven to nine times; their dimensions can permit an

intramolecular interaction between enzyme and inhibitor. In both organisms these proteins were subjected to proteolytic cleavage and this caused the release of active FpPG; the high residual polygalacturone activity caused severe growth defects in *Arabidopsis*. This effect was consistent with the one that had previously been observed in transgenic plants which expressed the PG of *Aspergillus niger*; such plants were not able to grow as a consequence of the high enzymatic activity present in the tissues (30). Subsequently, a fusion protein with a linker of only three alanine residues was generated; it was short enough not to permit intramolecular interactions, but capable of promoting intermolecular interactions between enzymes and inhibitors belonging to different chimeric molecules (FIG. **1***a*). When expressed in *P. pastoris*, the fusion protein was recovered as an intact polypeptide of the expected size, indicating a resistance to proteolysis (FIG. **4***a*). The protein was purified by affinity chromatography using the PGII of *A. niger* conjugated to a sepharose matrix, which was capable of binding the PGIP domain of the fusion protein (FIG. **5***b*, bottom panel). The enzymatic activity of the fusion protein was about 220 times lower than the FpPG (FIG. **4***b*, top panel). This suggested that the enzymatic activity had decreased markedly in the fusion protein due to the intramolecular interactions between the PG domain of one polypeptide and the PGIP domain of the other (FIG. **1***a*). Molecular crosslinking experiments confirmed this hypothesis and further revealed the ability of the chimera to bring about chain intermolecular interactions ranging from the dimer (~160 KDa) to the tetramer (320 KDa) (FIG. **4***a*), which were not observed when the FpPG or PvPGIP2 underwent crosslinking in the absence of the interaction partner (29). The construct PvPGIP2-FpPG was fused to the signal peptide of the bean PvPGIP2 to enable correct secretion in the cell wall (30) and it was later placed under the control of a β-estradiol-inducible promoter for stable expression in *Arabidopsis* (31). The presence of the protein in the leaves of the rosette was observed in the transgenic plants after 14 h of treatment with β-estradiol and it reached its maximum accumulation at 170 h after treatment (FIG. **1***b*). The accumulation was associated with the appearance of slight PG activity, which, as already previously demonstrated, is detected following the formation of the PG-PGIP molecular complex (4,6) (FIG. **1***b*). Adult transgenic plants did not show any obvious morphological defects when grown under normal conditions. However, following treatment with the inducer, the leaves of the transgenic plants showed discoloration and chlorosis starting from 170 h after the treatment (FIG. **1***d*). Treating the transgenic plants with an inducer also activated the defence responses typically induced following exogenous treatment with OGs, such as the expression of the marker genes for defence responses (RetOx and WRKY40; FIG. **1***e*) and the accumulation of callose (FIG. **10**. Taken as a whole, these results suggested that an accumulation of OGs might take place in β-estradiol-inducible transgenic plants that can be capable of activating defence responses similar to the ones induced as a result of an exogenous OG treatment. Because of this effect, the fusion protein was called "OG machine" (OGM). In order to verify whether the OGM actually caused the accumulation of OGs in the plant, pectin-enriched fractions were extracted from the leaves of the transgenic plants at 0, 24, 70 and 170 h following treatment with β-estradiol. The fractions were then analysed by high-performance anion exchange chromatography coupled with a pulsed amperometric detector (HPAEC-PAD), which revealed the presence of molecules with retention times comparable to those characterizing a mixture of OGs with a degree of polymerization comprised between 5 and 17; the concentration of these molecules increased with increases in the induction times (FIG. **2***a-d*). MALDI-TOF mass spectrometry also confirmed that these molecules were characterized by molecular masses corresponding to those of oligomers of unsubstituted polygalacturonic acid with a degree of polymerization of between 6 and 13 (FIG. **2***f*). Treatment of the fractions with a fully active PG of *A. niger* caused the molecules to disappear, confirming their OG nature (FIG. **5**).

Subsequently, we placed the transgene coding for the OGMs under the control of the terminal portion (1300 bps) of the promoter which regulates the expression of the gene PR-1 of *Arabidopsis* (PPR-1), strongly induced by bacterial and fungal infections (32-35). The construct (PPR1::OGM) was introduced in *Arabidopsis* and two independent transgenic lines were selected for a more thorough characterization. Neither transgenic plant showed any evident morphological difference compared to the wild plant Col-0 despite having, in the absence of the pathogen, a basal expression of the transgene that was greater in line 2 than in line 1. After inoculation with *Botrytis cinerea*, a significant increase (approximately 3-fold) was observed in the transcript coding for the OGM in both lines (FIG. **3***a*). The immunodecoration analysis confirmed the presence of a basal level of OGM in the uninfected plants, as well as the accumulation of the protein during infection with *B. cinerea* (FIG. **3***b*). Subsequently, we compared the susceptibility of the transgenic plants expressing the OGM and of the wild type plant Col-0 to some pathogenic microorganisms. The inocula of *B. cinerea* in the transgenic plants produced a reduced number of infections, whose success was indicated by the typical grey rot lesion (FIG. **3***c*). Moreover, the average area of the lesions in the plants of line 2 was significantly smaller than the one produced in wild type plants. The lesions produced in line 1 were likewise smaller than those of wild type plants, but this difference was not wholly significant (FIG. **3***d*). The transgenic plants also showed a marked resistance against the infections produced by *Pectobacterium carotovorum* (FIG. **3***e*) and *Pseudomonas syringae* pv. tomato DC3000. (FIG. **30**. In conclusion, the expression of the OGM under the control of a pathogen-inducible promoter seems to promote a non-specific resistance towards both fungal and bacterial pathogens, in which the release of OGs acts as a generic elicitor of defence responses. The OGM is characterised by a low residual enzymatic activity, which was optimal for regulating a controlled release of OGs in vivo and a controlled release of OGs in plants can in turn activate a wide range of defence responses, imparting resistance against pathogenic microorganisms to the plants.

Our research was mainly aimed at investigating the possible biotechnological applications of the OGM. The expression of the OGM under the control of a pathogen-inducible promoter enabled a rapid activation of defences which protected the transgenic plants against three major pathogens of agronomic interest. The present strategy of employing the OGM for the constitution of transgenic plants can be generally effective towards a broad range of pathogens and can represent a technology for protecting farm crops.

The controlled expression of the OGM following induction can be useful not only for engineering resistance, but also for studying the effects of OGs under physiological conditions. OGs activate defence responses on the one hand, and on the other hand they influence plant development and growth, acting like local auxin antagonists (36-40). The role of OGs as regulators of growth and development is mainly based on experiments that use exogenous OG treatments. To what degree OGs accumulate in intact tissues and in the

absence of a pathogen and how they act as endogenous regulators of plant growth and development can now be investigated.

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1 5 10 15

Ile Leu Val Ile Leu Val Ser Leu Ser Thr Ala His Ser Glu Leu Cys
20 25 30

Asn Pro Gln Asp Lys Gln Ala Leu Leu Gln Ile Lys Lys Asp Leu Gly
35 40 45

Asn Pro Thr Thr Leu Ser Ser Trp Leu Pro Thr Thr Asp Cys Cys Asn
50 55 60

Arg Thr Trp Leu Gly Val Leu Cys Asp Thr Asp Thr Gln Thr Tyr Arg
65 70 75 80

Val Asn Asn Leu Asp Leu Ser Gly Leu Asn Leu Pro Lys Pro Tyr Pro
85 90 95

Ile Pro Ser Ser Leu Ala Asn Leu Pro Tyr Leu Asn Phe Leu Tyr Ile
100 105 110

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Gly Gly Ile Asn Asn Leu Val Gly Pro Ile Pro Pro Ala Ile Ala Lys
        115                 120                 125

Leu Thr Gln Leu His Tyr Leu Tyr Ile Thr His Thr Asn Val Ser Gly
    130                 135                 140

Ala Ile Pro Asp Phe Leu Ser Gln Ile Lys Thr Leu Val Thr Leu Asp
145                 150                 155                 160

Phe Ser Tyr Asn Ala Leu Ser Gly Thr Leu Pro Pro Ser Ile Ser Ser
                165                 170                 175

Leu Pro Asn Leu Val Gly Ile Thr Phe Asp Gly Asn Arg Ile Ser Gly
            180                 185                 190

Ala Ile Pro Asp Ser Tyr Gly Ser Phe Ser Lys Leu Phe Thr Ser Met
        195                 200                 205

Thr Ile Ser Arg Asn Arg Leu Thr Gly Lys Ile Pro Pro Thr Phe Ala
    210                 215                 220

Asn Leu Asn Leu Ala Phe Val Asp Leu Ser Arg Asn Met Leu Glu Gly
225                 230                 235                 240

Asp Ala Ser Val Leu Phe Gly Ser Asp Lys Asn Thr Gln Lys Ile His
                245                 250                 255

Leu Ala Lys Asn Ser Leu Ala Phe Asp Leu Gly Lys Val Gly Leu Ser
            260                 265                 270

Lys Asn Leu Asn Gly Leu Asp Leu Arg Asn Asn Arg Ile Tyr Gly Thr
        275                 280                 285

Leu Pro Gln Gly Leu Thr Gln Leu Lys Phe Leu His Ser Leu Asn Val
    290                 295                 300

Ser Phe Asn Asn Leu Cys Gly Glu Ile Pro Gln Gly Gly Asn Leu Gln
305                 310                 315                 320

Arg Phe Asp Val Ser Ala Tyr Ala Asn Asn Lys Cys Leu Cys Gly Ser
                325                 330                 335

Pro Leu Pro Ala Cys Thr Ala Ala Ala Pro Cys Ser Val Thr Glu Tyr
            340                 345                 350

Ser Gly Leu Ala Thr Ala Val Ser Ser Cys Lys Asn Ile Val Leu Asn
        355                 360                 365

Gly Phe Gln Val Pro Thr Gly Lys Gln Leu Asp Leu Ser Ser Leu Gln
    370                 375                 380

Asn Asp Ser Thr Val Thr Phe Lys Gly Thr Thr Thr Phe Ala Thr Thr
385                 390                 395                 400

Ala Asp Asn Asp Phe Asn Pro Ile Val Ile Ser Gly Ser Asn Ile Thr
                405                 410                 415

Ile Thr Gly Ala Ser Gly His Val Ile Asp Gly Asn Gly Gln Ala Tyr
            420                 425                 430

Trp Asp Gly Lys Gly Ser Asn Ser Asn Ser Asn Gln Lys Pro Asp His
        435                 440                 445

Phe Ile Val Val Gln Lys Thr Thr Gly Asn Ser Lys Ile Thr Asn Leu
    450                 455                 460

Asn Ile Gln Asn Trp Pro Val His Cys Phe Asp Ile Thr Gly Ser Ser
465                 470                 475                 480

Gln Leu Thr Ile Ser Gly Leu Ile Leu Asp Asn Arg Ala Gly Asp Lys
                485                 490                 495

Pro Asn Ala Lys Ser Gly Ser Leu Pro Ala Ala His Asn Thr Asp Gly
            500                 505                 510

Phe Asp Ile Ser Ser Ser Asp His Val Thr Leu Asp Asn Asn His Val
        515                 520                 525
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Tyr Asn Gln Asp Asp Cys Val Ala Val Thr Ser Gly Thr Asn Ile Val
    530                 535                 540

Val Ser Asn Met Tyr Cys Ser Gly Gly His Gly Leu Ser Ile Gly Ser
545                 550                 555                 560

Val Gly Gly Lys Ser Asp Asn Val Val Asp Gly Val Gln Phe Leu Ser
                565                 570                 575

Ser Gln Val Val Asn Ser Gln Asn Gly Cys Arg Ile Lys Ser Asn Ser
            580                 585                 590

Gly Ala Thr Gly Thr Ile Asn Asn Val Thr Tyr Gln Asn Ile Ala Leu
        595                 600                 605

Thr Asn Ile Ser Thr Tyr Gly Val Asp Val Gln Gln Asp Tyr Leu Asn
    610                 615                 620

Gly Gly Pro Thr Gly Lys Pro Thr Asn Gly Val Lys Ile Ser Asn Ile
625                 630                 635                 640

Lys Phe Ile Lys Val Thr Gly Thr Val Ala Ser Ser Ala Gln Asp Trp
                645                 650                 655

Phe Ile Leu Cys Gly Asp Gly Ser Cys Ser Gly Phe Thr Phe Ser Gly
            660                 665                 670

Asn Ala Ile Thr Gly Gly Gly Lys Thr Ser Ser Cys Asn Tyr Pro Thr
        675                 680                 685

Asn Thr Cys Pro Ser
    690
```

<210> SEQ ID NO 7
<211> LENGTH: 1995
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of OGM expressed in Pichia

<400> SEQUENCE: 7

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gagctatgca acccacaaga caagcaagcc cttctccaaa tcaagaaaga ccttggcaac     60

ccaaccactc tctcctcatg gcttccaacc accgactgtt gcaacagaac ctggctaggt    120

gttttatgcg acaccgacac ccaaacatat cgcgtcaaca acctcgacct ctccggcctt    180

aacctcccaa aaccctaccc tatcccttcc tccctcgcca acctccccta cctcaatttt    240

ctatacattg gtggcatcaa taacctcgtc ggtccaatcc cccccgccat cgctaaactc    300

acccaactcc actatctcta tatcacccac accaatgtct ccggcgcaat acccgatttc    360

ttgtcacaga tcaaaaccct cgtcaccctc gacttctcct acaacgccct ctccggcacc    420

ctacctccct ccatctcttc tctccccaac ctcgtcggaa tcacattcga cggcaaccga    480

atctccggcg ccatccccga ctcctacggc tcattttcga agctgttcac gtcgatgacc    540

atctcccgca accgcctcac cgggaagatt ccgccgacgt ttgcgaatct gaacctggcg    600

ttcgttgact tgtctcgaaa catgctggag ggtgacgcgt cggtgttgtt cggatcagat    660

aagaacacgc agaagataca tctggcgaag aactctcttg cctttgattt ggggaaagtg    720

gggttgtcaa agaacttgaa cgggttggat ctgaggaaca accgtatcta tgggacgcta    780

ccgcagggac tgacgcagct aaagtttctg cacagtttaa atgtgagctt caacaatctg    840

tgcggtgaga ttcctcaagg tgggaacttg caaagatttg acgtttctgc ttatgccaac    900

aacaagtgct tgtgtggttc tcctcttcct gcctgcactg cggccgcacc ctgctccgtg    960

actgagtact ctggcctcgc caccgctgtc tcatcctgca aaaacatcgt gctcaacggt   1020

ttccaagtcc cgacaggcaa gcaactcgac ctatccagcc tccagaatga ctcgaccgtt   1080
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accttcaagg gcacgaccac ttttgccacc actgctgata acgactttaa tcctatcgtc 1140

attagtggaa gtaacatcac tatcactggt gcatctggcc atgtcattga tggcaacggt 1200

caggcgtact gggatggcaa aggttctaac agcaatagca accaaaagcc cgatcacttc 1260

atcgttgttc agaagaccac cggcaactca aagatcacaa acctaaatat ccagaactgg 1320

cccgttcact gcttcgacat tacaggcagc tcgcaattga ccatctcagg gcttattctt 1380

gataacagag ctggcgacaa gcctaacgcc aagagcggta gcttgcccgc tgcgcataac 1440

accgacggtt tcgacatctc gtccagtgac cacgttacgc tggataacaa tcatgtttat 1500

aaccaagatg attgtgttgc tgttacttcc ggtacaaaca tcgtcgtttc taacatgtat 1560

tgctccggcg gccatggtct tagtatcgga tctgttggtg gaaagagcga caatgtcgtt 1620

gatggtgttc agttcttgag ctcgcaggtt gtgaacagtc agaatggatg tcgcatcaag 1680

tccaactctg gcgcaactgg cacgatcaac aacgtcacct accagaacat tgctctcacc 1740

aacatcagca cgtacggtgt cgatgttcag caggactatc tcaacggcgg ccctactgga 1800

aagccgacca acggagtcaa gatcagcaac atcaagttca tcaaggtcac tggcactgtg 1860

gctagctctg cccaggattg gtttattctg tgtggtgatg gtagctgctc tggatttacc 1920

ttctctggaa acgctattac tggtggtggc aagactagca gctgcaacta tcctaccaac 1980

acttgcccca gctag 1995

<210> SEQ ID NO 8
<211> LENGTH: 664
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of OGM expressed in Pichia

<400> SEQUENCE: 8

Glu Leu Cys Asn Pro Gln Asp Lys Gln Ala Leu Leu Gln Ile Lys Lys
1 5 10 15

Asp Leu Gly Asn Pro Thr Thr Leu Ser Ser Trp Leu Pro Thr Thr Asp
20 25 30

Cys Cys Asn Arg Thr Trp Leu Gly Val Leu Cys Asp Thr Asp Thr Gln
35 40 45

Thr Tyr Arg Val Asn Asn Leu Asp Leu Ser Gly Leu Asn Leu Pro Lys
50 55 60

Pro Tyr Pro Ile Pro Ser Ser Leu Ala Asn Leu Pro Tyr Leu Asn Phe
65 70 75 80

Leu Tyr Ile Gly Gly Ile Asn Asn Leu Val Gly Pro Ile Pro Pro Ala
85 90 95

Ile Ala Lys Leu Thr Gln Leu His Tyr Leu Tyr Ile Thr His Thr Asn
100 105 110

Val Ser Gly Ala Ile Pro Asp Phe Leu Ser Gln Ile Lys Thr Leu Val
115 120 125

Thr Leu Asp Phe Ser Tyr Asn Ala Leu Ser Gly Thr Leu Pro Pro Ser
130 135 140

Ile Ser Ser Leu Pro Asn Leu Val Gly Ile Thr Phe Asp Gly Asn Arg
145 150 155 160

Ile Ser Gly Ala Ile Pro Asp Ser Tyr Gly Ser Phe Ser Lys Leu Phe
165 170 175

Thr Ser Met Thr Ile Ser Arg Asn Arg Leu Thr Gly Lys Ile Pro Pro
180 185 190

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Thr Phe Ala Asn Leu Asn Leu Ala Phe Val Asp Leu Ser Arg Asn Met
        195                 200                 205

Leu Glu Gly Asp Ala Ser Val Leu Phe Gly Ser Asp Lys Asn Thr Gln
    210                 215                 220

Lys Ile His Leu Ala Lys Asn Ser Leu Ala Phe Asp Leu Gly Lys Val
225                 230                 235                 240

Gly Leu Ser Lys Asn Leu Asn Gly Leu Asp Leu Arg Asn Asn Arg Ile
                245                 250                 255

Tyr Gly Thr Leu Pro Gln Gly Leu Thr Gln Leu Lys Phe Leu His Ser
            260                 265                 270

Leu Asn Val Ser Phe Asn Asn Leu Cys Gly Glu Ile Pro Gln Gly Gly
        275                 280                 285

Asn Leu Gln Arg Phe Asp Val Ser Ala Tyr Ala Asn Asn Lys Cys Leu
    290                 295                 300

Cys Gly Ser Pro Leu Pro Ala Cys Thr Ala Ala Ala Pro Cys Ser Val
305                 310                 315                 320

Thr Glu Tyr Ser Gly Leu Ala Thr Ala Val Ser Ser Cys Lys Asn Ile
                325                 330                 335

Val Leu Asn Gly Phe Gln Val Pro Thr Gly Lys Gln Leu Asp Leu Ser
            340                 345                 350

Ser Leu Gln Asn Asp Ser Thr Val Thr Phe Lys Gly Thr Thr Thr Phe
        355                 360                 365

Ala Thr Thr Ala Asp Asn Asp Phe Asn Pro Ile Val Ile Ser Gly Ser
    370                 375                 380

Asn Ile Thr Ile Thr Gly Ala Ser Gly His Val Ile Asp Gly Asn Gly
385                 390                 395                 400

Gln Ala Tyr Trp Asp Gly Lys Gly Ser Asn Ser Asn Ser Asn Gln Lys
                405                 410                 415

Pro Asp His Phe Ile Val Val Gln Lys Thr Thr Gly Asn Ser Lys Ile
            420                 425                 430

Thr Asn Leu Asn Ile Gln Asn Trp Pro Val His Cys Phe Asp Ile Thr
        435                 440                 445

Gly Ser Ser Gln Leu Thr Ile Ser Gly Leu Ile Leu Asp Asn Arg Ala
    450                 455                 460

Gly Asp Lys Pro Asn Ala Lys Ser Gly Ser Leu Pro Ala Ala His Asn
465                 470                 475                 480

Thr Asp Gly Phe Asp Ile Ser Ser Ser Asp His Val Thr Leu Asp Asn
                485                 490                 495

Asn His Val Tyr Asn Gln Asp Asp Cys Val Ala Val Thr Ser Gly Thr
            500                 505                 510

Asn Ile Val Val Ser Asn Met Tyr Cys Ser Gly Gly His Gly Leu Ser
        515                 520                 525

Ile Gly Ser Val Gly Gly Lys Ser Asp Asn Val Val Asp Gly Val Gln
    530                 535                 540

Phe Leu Ser Ser Gln Val Val Asn Ser Gln Asn Gly Cys Arg Ile Lys
545                 550                 555                 560

Ser Asn Ser Gly Ala Thr Gly Thr Ile Asn Asn Val Thr Tyr Gln Asn
                565                 570                 575

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

Tyr Leu Asn Gly Gly Pro Thr Gly Lys Pro Thr Asn Gly Val Lys Ile
        595                 600                 605
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Ser Asn Ile Lys Phe Ile Lys Val Thr Gly Thr Val Ala Ser Ser Ala
    610                 615                 620

Gln Asp Trp Phe Ile Leu Cys Gly Asp Gly Ser Cys Ser Gly Phe Thr
625                 630                 635                 640

Phe Ser Gly Asn Ala Ile Thr Gly Gly Gly Lys Thr Ser Ser Cys Asn
                645                 650                 655

Tyr Pro Thr Asn Thr Cys Pro Ser
            660


<210> SEQ ID NO 9
<211> LENGTH: 3373
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of OGM fused to promoter
      PR-1

<400> SEQUENCE: 9

aagcttgttt taacttataa aatgattctc cctccatata aaaaagtttg attttataga     60

atgtttatac cgattaaaaa aataataatg cttagttata aattactatt tattcatgct    120

aaactatttc tcgtaactat taaccaatag taattcatca aattttaaaa ttctcaatta    180

attgattctt gaaattcata acctttttaat attgattgat aaaaatatac ataaactcaa    240

tcttttttaat acaaaaaaac tttaaaaaat caatttttct gattcggagg gagtatatgt    300

tattgcttag aatcacagat tcatatcagg attggaaaat tttaaagcca gtgcatatca    360

gtagtcaaaa ttggtaaatg atatacgaag gcggtacaaa attaggtata ctgaagatag    420

aagaacacaa aagtagatcg gtcacctaga gtttttcaat ttaaactgcg tattagtgtt    480

tggaaaaaaa aaacaaagtg tatacaatgt caatcggtga tctttttttt ttttttttt    540

ttttttttc tttttggata aatctcaatg ggtgatctat tgactgtttc tctacgtcac    600

tattttactt acgtcataga tgtggcggca tatattcttc aggacttttc agccataggc    660

aagagtgata gagatactca tatgcatgaa acactaagaa acaaataatt cttgactttt    720

tttcttttat ttgaaaattg actgtagata taaactttta ttttttctga ctgtaaatat    780

aatcttaatt gccaaactgt ccgatacgat ttttctgtat tatttacagg aagatatctt    840

caaaacattt tgaatgaagt aatatatgaa attcaaattt gaaatagaag acttaaatta    900

gaatcatgaa gaaaaaaaaa acacaaaaca actgaatgac atgaaacaac tatatacaat    960

gtttcttaat aaacttcatt tagggtatac ttacatatat actaaaaaaa tatatcaaca   1020

atggcaaagc taccgatacg aaacaatatt aggaaaaatg tgtgtaagga caagattgac   1080

aaaaaaatag ttacgaaaac aacttctatt catttggaca attgcaatga atattactaa   1140

aatactcaca catggaccat gtatttacaa aaacgtgaga tctatagtta acaaaaaaaa   1200

aaagaaaaaa atagttttca aatctctata taagcgatgt ttacgaaccc caaaatcata   1260

acacaacaat aaccattatc aacttagaaa aatgactcaa ttcaatatcc cagtaaccat   1320

gtcttcaagc ttaagcataa ttttggtcat tcttgtatct ttgagcactg cacactcaga   1380

gctatgcaac ccacaagaca agcaagccct tctccaaatc aagaaagacc ttggcaaccc   1440

aaccactctc tcctcatggc ttccaaccac cgactgttgc aacagaacct ggctaggtgt   1500

tttatgcgac accgacaccc aaacatatcg cgtcaacaac ctcgacctct ccggccttaa   1560

cctcccaaaa ccctacccta tcccttcctc cctcgccaac ctcccctacc tcaattttct   1620

atacattggt ggcatcaata acctcgtcgg tccaatcccc cccgccatcg ctaaactcac   1680
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ccaactccac tatctctata tcacccacac caatgtctcc ggcgcaatac ccgatttctt 1740

gtcacagatc aaaaccctcg tcaccctcga cttctcctac aacgccctct ccggcaccct 1800

acctccctcc atctcttctc tccccaacct cgtcggaatc acattcgacg gcaaccgaat 1860

ctccggcgcc atccccgact cctacggctc attttcgaag ctgttcacgt cgatgaccat 1920

ctcccgcaac cgcctcaccg ggaagattcc gccgacgttt gcgaatctga acctggcgtt 1980

cgttgacttg tctcgaaaca tgctggaggg tgacgcgtcg gtgttgttcg gatcagataa 2040

gaacacgcag aagatacatc tggcgaagaa ctctcttgcc tttgatttgg ggaaagtggg 2100

gttgtcaaag aacttgaacg ggttggatct gaggaacaac cgtatctatg ggacgctacc 2160

gcagggactg acgcagctaa agtttctgca cagtttaaat gtgagcttca acaatctgtg 2220

cggtgagatt cctcaaggtg ggaacttgca aagatttgac gtttctgctt atgccaacaa 2280

caagtgcttg tgtggttctc ctcttcctgc ctgcactgcg gccgcaccct gctccgtgac 2340

tgagtactct ggcctcgcca ccgctgtctc atcctgcaaa aacatcgtgc tcaacggttt 2400

ccaagtcccg acaggcaagc aactcgacct atccagcctc cagaatgact cgaccgttac 2460

cttcaagggc acgaccactt ttgccaccac tgctgataac gactttaatc ctatcgtcat 2520

tagtggaagt aacatcacta tcactggtgc atctggccat gtcattgatg gcaacggtca 2580

ggcgtactgg gatggcaaag gttctaacag caatagcaac caaaagcccg atcacttcat 2640

cgttgttcag aagaccaccg gcaactcaaa gatcacaaac ctaaatatcc agaactggcc 2700

cgttcactgc ttcgacatta caggcagctc gcaattgacc atctcagggc ttattcttga 2760

taacagagct ggcgacaagc ctaacgccaa gagcggtagc ttgcccgctg cgcataacac 2820

cgacggtttc gacatctcgt ccagtgacca cgttacgctg gataacaatc atgtttataa 2880

ccaagatgat tgtgttgctg ttacttccgg tacaaacatc gtcgtttcta acatgtattg 2940

ctccggcggc catggtctta gtatcggatc tgttggtgga aagagcgaca atgtcgttga 3000

tggtgttcag ttcttgagct cgcaggttgt gaacagtcag aatggatgtc gcatcaagtc 3060

caactctggc gcaactggca cgatcaacaa cgtcacctac cagaacattg ctctcaccaa 3120

catcagcacg tacggtgtcg atgttcagca ggactatctc aacggcggcc ctactggaaa 3180

gccgaccaac ggagtcaaga tcagcaacat caagttcatc aaggtcactg gcactgtggc 3240

tagctctgcc caggattggt ttattctgtg tggtgatggt agctgctctg gatttacctt 3300

ctctggaaac gctattactg gtggtggcaa gactagcagc tgcaactatc ctaccaacac 3360

ttgccccagc tag 3373

<210> SEQ ID NO 10
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 10

Ala Thr Cys Gly Ala Thr Gly Ala Ala Thr Thr Cys Gly Ala Gly Cys
1 5 10 15

Thr Ala Thr Gly Cys Ala Ala Cys Cys Cys Ala Cys Ala
20 25

<210> SEQ ID NO 11
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 11

tcttctaagt gcggccgcag tgcaggcagg aagag 35

<210> SEQ ID NO 12
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 12

tcaacactat gcggccgcac cctgctccgt gactgag 37

<210> SEQ ID NO 13
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 13

atcgattcta gactagctgg ggcaagtgtt g 31

<210> SEQ ID NO 14
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 14

actaagccta ggactatcta gaatgactca attcaatatc ccag 44

<210> SEQ ID NO 15
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 15

ggggatggcg ccggag 16

<210> SEQ ID NO 16
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 16

actaagctcg agatgactca attcaatatc ccag 34

<210> SEQ ID NO 17
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 17

cctaagttaa ttaactagct ggggcaagtg ttg 33

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<210> SEQ ID NO 18
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 18

gactatctag aatgactcaa ttcaatatcc c 31

<210> SEQ ID NO 19
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 19

gttagcacaa gcttgtttta ac 22

<210> SEQ ID NO 20
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 20

cctaagtcta gaggtcttaa ttaactagct gggg 34

<210> SEQ ID NO 21
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 21

tgcttaagct tgaagacatg gttactggga tattgaattg agtcattttt ctaagttgat 60

aatgg 65

<210> SEQ ID NO 22
<211> LENGTH: 373
<212> TYPE: PRT
<213> ORGANISM: Fusarium phyllophilum

<400> SEQUENCE: 22

Met Val Arg Asn Ile Val Ser Arg Leu Cys Ser Gln Leu Phe Ala Leu
1 5 10 15

Pro Ser Ser Ser Leu Gln Glu Arg Asp Pro Cys Ser Val Thr Glu Tyr
20 25 30

Ser Gly Leu Ala Thr Ala Val Ser Ser Cys Lys Asn Ile Val Leu Asn
35 40 45

Gly Phe Gln Val Pro Thr Gly Lys Gln Leu Asp Leu Ser Ser Leu Gln
50 55 60

Asn Asp Ser Thr Val Thr Phe Lys Gly Thr Thr Thr Phe Ala Thr Thr
65 70 75 80

Ala Asp Asn Asp Phe Asn Pro Ile Val Ile Ser Gly Ser Asn Ile Thr
85 90 95

Ile Thr Gly Ala Ser Gly His Val Ile Asp Gly Asn Gly Gln Ala Tyr
100 105 110

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Trp Asp Gly Lys Gly Ser Asn Ser Asn Ser Asn Gln Lys Pro Asp His
        115                 120                 125

Phe Ile Val Val Gln Lys Thr Thr Gly Asn Ser Lys Ile Thr Asn Leu
    130                 135                 140

Asn Ile Gln Asn Trp Pro Val His Cys Phe Asp Ile Thr Gly Ser Ser
145                 150                 155                 160

Gln Leu Thr Ile Ser Gly Leu Ile Leu Asp Asn Arg Ala Gly Asp Lys
                165                 170                 175

Pro Asn Ala Lys Ser Gly Ser Leu Pro Ala Ala His Asn Thr Asp Gly
            180                 185                 190

Phe Asp Ile Ser Ser Ser Asp His Val Thr Leu Asp Asn Asn His Val
        195                 200                 205

Tyr Asn Gln Asp Asp Cys Val Ala Val Thr Ser Gly Thr Asn Ile Val
    210                 215                 220

Val Ser Asn Met Tyr Cys Ser Gly Gly His Gly Leu Ser Ile Gly Ser
225                 230                 235                 240

Val Gly Gly Lys Ser Asp Asn Val Val Asp Gly Val Gln Phe Leu Ser
                245                 250                 255

Ser Gln Val Val Asn Ser Gln Asn Gly Cys Arg Ile Lys Ser Asn Ser
            260                 265                 270

Gly Ala Thr Gly Thr Ile Asn Asn Val Thr Tyr Gln Asn Ile Ala Leu
        275                 280                 285

Thr Asn Ile Ser Thr Tyr Gly Val Asp Val Gln Gln Asp Tyr Leu Asn
    290                 295                 300

Gly Gly Pro Thr Gly Lys Pro Thr Asn Gly Val Lys Ile Ser Asn Ile
305                 310                 315                 320

Lys Phe Ile Lys Val Thr Gly Thr Val Ala Ser Ser Ala Gln Asp Trp
                325                 330                 335

Phe Ile Leu Cys Gly Asp Gly Ser Cys Ser Gly Phe Thr Phe Ser Gly
            340                 345                 350

Asn Ala Ile Thr Gly Gly Gly Lys Thr Ser Ser Cys Asn Tyr Pro Thr
        355                 360                 365

Asn Thr Cys Pro Ser
    370


<210> SEQ ID NO 23
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Phaseolus vulgaris

<400> SEQUENCE: 23

Met Thr Gln Phe Asn Ile Pro Val Thr Met Ser Ser Ser Leu Ser Ile
1               5                   10                  15

Ile Leu Val Ile Leu Val Ser Leu Arg Thr Ala Leu Ser Glu Leu Cys
            20                  25                  30

Asn Pro Gln Asp Lys Gln Ala Leu Leu Gln Ile Lys Lys Asp Leu Gly
        35                  40                  45

Asn Pro Thr Thr Leu Ser Ser Trp Leu Pro Thr Thr Asp Cys Cys Asn
    50                  55                  60

Arg Thr Trp Leu Gly Val Leu Cys Asp Thr Asp Thr Gln Thr Tyr Arg
65                  70                  75                  80

Val Asn Asn Leu Asp Leu Ser Gly His Asn Leu Pro Lys Pro Tyr Pro
                85                  90                  95

Ile Pro Ser Ser Leu Ala Asn Leu Pro Tyr Leu Asn Phe Leu Tyr Ile
            100                 105                 110
```

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```
Gly Gly Ile Asn Asn Leu Val Gly Pro Ile Pro Pro Ala Ile Ala Lys
        115                 120                 125

Leu Thr Gln Leu His Tyr Leu Tyr Ile Thr His Thr Asn Val Ser Gly
    130                 135                 140

Ala Ile Pro Asp Phe Leu Ser Gln Ile Lys Thr Leu Val Thr Leu Asp
145                 150                 155                 160

Phe Ser Tyr Asn Ala Leu Ser Gly Thr Leu Pro Pro Ser Ile Ser Ser
                165                 170                 175

Leu Pro Asn Leu Gly Gly Ile Thr Phe Asp Gly Asn Arg Ile Ser Gly
            180                 185                 190

Ala Ile Pro Asp Ser Tyr Gly Ser Phe Ser Lys Leu Phe Thr Ala Met
        195                 200                 205

Thr Ile Ser Arg Asn Arg Leu Thr Gly Lys Ile Pro Pro Thr Phe Ala
    210                 215                 220

Asn Leu Asn Leu Ala Phe Val Asp Leu Ser Arg Asn Met Leu Glu Gly
225                 230                 235                 240

Asp Ala Ser Val Leu Phe Gly Ser Asp Lys Asn Thr Lys Lys Ile His
                245                 250                 255

Leu Ala Lys Asn Ser Leu Ala Phe Asp Leu Gly Lys Val Gly Leu Ser
            260                 265                 270

Lys Asn Leu Asn Gly Leu Asp Leu Arg Asn Asn Arg Ile Tyr Gly Thr
        275                 280                 285

Leu Pro Gln Gly Leu Thr Gln Leu Lys Phe Leu Gln Ser Leu Asn Val
    290                 295                 300

Ser Phe Asn Asn Leu Cys Gly Glu Ile Pro Gln Gly Gly Asn Leu Lys
305                 310                 315                 320

Arg Phe Asp Val Ser Ser Tyr Ala Asn Asn Lys Cys Leu Cys Gly Ser
                325                 330                 335

Pro Leu Pro Ser Cys Thr
            340


<210> SEQ ID NO 24
<211> LENGTH: 362
<212> TYPE: PRT
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 24

Met His Ser Phe Ala Ser Leu Leu Ala Tyr Gly Leu Val Ala Gly Ala
1               5                   10                  15

Thr Phe Ala Ser Ala Ser Pro Ile Glu Ala Arg Asp Ser Cys Thr Phe
            20                  25                  30

Thr Thr Ala Ala Ala Ala Lys Ala Gly Lys Ala Lys Cys Ser Thr Ile
        35                  40                  45

Thr Leu Asn Asn Ile Glu Val Pro Ala Gly Thr Thr Leu Asp Leu Thr
    50                  55                  60

Gly Leu Thr Ser Gly Thr Lys Val Ile Phe Glu Gly Thr Thr Thr Phe
65                  70                  75                  80

Gln Tyr Glu Glu Trp Ala Gly Pro Leu Ile Ser Met Ser Gly Glu His
                85                  90                  95

Ile Thr Val Thr Gly Ala Ser Gly His Leu Ile Asn Cys Asp Gly Ala
            100                 105                 110

Arg Trp Trp Asp Gly Lys Gly Thr Ser Gly Lys Lys Lys Pro Lys Phe
        115                 120                 125

Phe Tyr Ala His Gly Leu Asp Ser Ser Ser Ile Thr Gly Leu Asn Ile
    130                 135                 140
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```
Lys Asn Thr Pro Leu Met Ala Phe Ser Val Gln Ala Asn Asp Ile Thr
145                 150                 155                 160

Phe Thr Asp Val Thr Ile Asn Asn Ala Asp Gly Asp Thr Gln Gly Gly
                165                 170                 175

His Asn Thr Asp Ala Phe Asp Val Gly Asn Ser Val Gly Val Asn Ile
            180                 185                 190

Ile Lys Pro Trp Val His Asn Gln Asp Asp Cys Leu Ala Val Asn Ser
        195                 200                 205

Gly Glu Asn Ile Trp Phe Thr Gly Gly Thr Cys Ile Gly Gly His Gly
    210                 215                 220

Leu Ser Ile Gly Ser Val Gly Asp Arg Ser Asn Asn Val Val Lys Asn
225                 230                 235                 240

Val Thr Ile Glu His Ser Thr Val Ser Asn Ser Glu Asn Ala Val Arg
                245                 250                 255

Ile Lys Thr Ile Ser Gly Ala Thr Gly Ser Val Ser Glu Ile Thr Tyr
            260                 265                 270

Ser Asn Ile Val Met Ser Gly Ile Ser Asp Tyr Gly Val Val Ile Gln
        275                 280                 285

Gln Asp Tyr Glu Asp Gly Lys Pro Thr Gly Lys Pro Thr Asn Gly Val
    290                 295                 300

Thr Ile Gln Asp Val Lys Leu Glu Ser Val Thr Gly Ser Val Asp Ser
305                 310                 315                 320

Gly Ala Thr Glu Ile Tyr Leu Leu Cys Gly Ser Gly Ser Cys Ser Asp
                325                 330                 335

Trp Thr Trp Asp Asp Val Lys Val Thr Gly Gly Lys Lys Ser Thr Ala
            340                 345                 350

Cys Lys Asn Phe Pro Ser Val Ala Ser Cys
        355                 360


<210> SEQ ID NO 25
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Phaseolus vulgaris

<400> SEQUENCE: 25

Met Thr Gln Phe Asn Ile Pro Val Thr Met Ser Ser Ser Leu Ser Ile
1               5                   10                  15

Ile Leu Val Ile Leu Val Ser Leu Arg Thr Ala Leu Ser Glu Leu Cys
            20                  25                  30

Asn Pro Gln Asp Lys Gln Ala Leu Leu Gln Ile Lys Lys Asp Leu Gly
        35                  40                  45

Asn Pro Thr Thr Leu Ser Ser Trp Leu Pro Thr Thr Asp Cys Cys Asn
    50                  55                  60

Arg Thr Trp Leu Gly Val Leu Cys Asp Thr Asp Thr Gln Thr Tyr Arg
65                  70                  75                  80

Val Asn Asn Leu Asp Leu Ser Gly His Asn Leu Pro Lys Pro Tyr Pro
                85                  90                  95

Ile Pro Ser Ser Leu Ala Asn Leu Pro Tyr Leu Asn Phe Leu Tyr Ile
            100                 105                 110

Gly Gly Ile Asn Asn Leu Val Gly Pro Ile Pro Pro Ala Ile Ala Lys
        115                 120                 125

Leu Thr Gln Leu His Tyr Leu Tyr Ile Thr His Thr Asn Val Ser Gly
    130                 135                 140

Ala Ile Pro Asp Phe Leu Ser Gln Ile Lys Thr Leu Val Thr Leu Asp
145                 150                 155                 160
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Phe Ser Tyr Asn Ala Leu Ser Gly Thr Leu Pro Pro Ser Ile Ser Ser
165 170 175

Leu Pro Asn Leu Val Gly Ile Thr Phe Asp Gly Asn Arg Ile Ser Gly
180 185 190

Ala Ile Pro Asp Ser Tyr Gly Ser Phe Ser Lys Leu Phe Thr Ser Met
195 200 205

Thr Ile Ser Arg Asn Arg Leu Thr Gly Lys Ile Pro Pro Thr Phe Ala
210 215 220

Asn Leu Asn Leu Ala Phe Val Asp Leu Ser Arg Asn Met Leu Gln Gly
225 230 235 240

Asp Ala Ser Val Leu Phe Gly Ser Asp Lys Asn Thr Gln Lys Ile His
245 250 255

Leu Ala Lys Asn Ser Leu Asp Phe Asp Leu Glu Lys Val Gly Leu Ser
260 265 270

Lys Asn Leu Asn Gly Leu Asp Leu Arg Asn Asn Arg Ile Tyr Gly Thr
275 280 285

Leu Pro Gln Gly Leu Thr Gln Leu Lys Phe Leu His Ser Leu Asn Val
290 295 300

Ser Phe Asn Asn Leu Cys Gly Glu Ile Pro Gln Gly Gly Asn Leu Gln
305 310 315 320

Arg Phe Asp Val Ser Ala Tyr Ala Asn Asn Lys Cys Leu Cys Gly Ser
325 330 335

Pro Leu Pro Ala Cys Thr
340

<210> SEQ ID NO 26
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Malus domestica

<400> SEQUENCE: 26

Met Glu Leu Lys Phe Ser Ile Phe Leu Ser Leu Thr Leu Leu Phe Ser
1 5 10 15

Ser Val Leu Lys Pro Ala Leu Ser Asp Leu Cys Asn Pro Asp Asp Lys
20 25 30

Lys Val Leu Leu Gln Ile Lys Lys Ala Phe Gly Asp Pro Tyr Val Leu
35 40 45

Thr Ser Trp Lys Ser Asp Thr Asp Cys Cys Asp Trp Tyr Cys Val Thr
50 55 60

Cys Asp Ser Thr Thr Asn Arg Ile Asn Ser Leu Thr Ile Phe Ala Gly
65 70 75 80

Gln Val Ser Gly Gln Ile Pro Ala Leu Val Gly Asp Leu Pro Tyr Leu
85 90 95

Glu Thr Leu Glu Phe His Lys Gln Pro Asn Leu Thr Gly Pro Ile Gln
100 105 110

Pro Ala Ile Ala Lys Leu Lys Gly Leu Lys Phe Leu Arg Leu Ser Trp
115 120 125

Thr Asn Leu Ser Gly Ser Val Pro Asp Phe Leu Ser Gln Leu Lys Asn
130 135 140

Leu Thr Phe Leu Asp Leu Ser Phe Asn Asn Leu Thr Gly Ala Ile Pro
145 150 155 160

Ser Ser Leu Ser Gln Leu Pro Asn Leu Asn Ala Leu His Leu Asp Arg
165 170 175

Asn Lys Leu Thr Gly His Ile Pro Lys Ser Leu Gly Gln Phe Ile Gly
180 185 190

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```
Asn Val Pro Asp Leu Tyr Leu Ser His Asn Gln Leu Ser Gly Asn Ile
        195                 200                 205

Pro Thr Ser Phe Ala Gln Met Asp Phe Thr Ser Ile Asp Leu Ser Arg
    210                 215                 220

Asn Lys Leu Glu Gly Asp Ala Ser Val Ile Phe Gly Leu Asn Lys Thr
225                 230                 235                 240

Thr Gln Ile Val Asp Leu Ser Arg Asn Leu Leu Glu Phe Asn Leu Ser
                245                 250                 255

Lys Val Glu Phe Pro Thr Ser Leu Thr Ser Leu Asp Ile Asn His Asn
            260                 265                 270

Lys Ile Tyr Gly Ser Ile Pro Val Glu Phe Thr Gln Leu Asn Phe Gln
        275                 280                 285

Phe Leu Asn Val Ser Tyr Asn Arg Leu Cys Gly Gln Ile Pro Val Gly
    290                 295                 300

Gly Lys Leu Gln Ser Phe Asp Glu Tyr Ser Tyr Phe His Asn Arg Cys
305                 310                 315                 320

Leu Cys Gly Ala Pro Leu Pro Ser Cys Lys
                325                 330


<210> SEQ ID NO 27
<211> LENGTH: 362
<212> TYPE: PRT
<213> ORGANISM: Colletotrichum lupini var setosum

<400> SEQUENCE: 27

Met Val Ser Ser Leu Leu Ala Leu Gly Ala Leu Ala Ala Thr Ala Ile
1               5                   10                  15

Ala Ala Pro Leu Asp Ala Arg Ala Ser Cys Thr Phe Thr Asp Ala Ala
            20                  25                  30

Ala Ala Ile Lys Gly Lys Ala Ser Cys Thr Ser Ile Ile Leu Asn Gly
        35                  40                  45

Ile Val Val Pro Ala Gly Thr Thr Leu Asp Met Thr Gly Leu Lys Ser
    50                  55                  60

Gly Thr Thr Val Thr Phe Gln Gly Lys Thr Thr Phe Gly Tyr Lys Glu
65                  70                  75                  80

Trp Glu Gly Pro Leu Ile Ser Phe Ser Gly Thr Asn Ile Asn Ile Asn
                85                  90                  95

Gly Ala Ser Gly His Ser Ile Asp Cys Gln Gly Ser Arg Trp Trp Asp
            100                 105                 110

Ser Lys Gly Ser Asn Gly Gly Lys Thr Lys Pro Lys Phe Phe Tyr Ala
        115                 120                 125

His Ser Leu Lys Ser Ser Asn Ile Lys Gly Leu Asn Val Leu Asn Thr
    130                 135                 140

Pro Val Gln Ala Phe Ser Ile Asn Ser Ala Thr Thr Leu Gly Val Tyr
145                 150                 155                 160

Asp Val Ile Ile Asp Asn Ser Ala Gly Asp Ser Ala Gly Gly His Asn
                165                 170                 175

Thr Asp Ala Phe Asp Val Gly Ser Ser Thr Gly Val Tyr Ile Ser Gly
            180                 185                 190

Ala Asn Val Lys Asn Gln Asp Asp Cys Leu Ala Ile Asn Ser Gly Thr
        195                 200                 205

Asn Ile Thr Phe Thr Gly Gly Thr Cys Ser Gly Gly His Gly Leu Ser
    210                 215                 220

Ile Gly Ser Val Gly Gly Arg Ser Asp Asn Thr Val Lys Thr Val Thr
225                 230                 235                 240
```

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```
Ile Ser Asn Ser Lys Ile Val Asn Ser Asp Asn Gly Val Arg Ile Lys
                245                 250                 255

Thr Val Ser Gly Ala Thr Gly Ser Val Ser Gly Val Thr Tyr Ser Gly
            260                 265                 270

Ile Thr Leu Ser Asn Ile Ala Lys Tyr Gly Ile Val Ile Glu Gln Asp
        275                 280                 285

Tyr Glu Asn Gly Ser Pro Thr Gly Thr Pro Thr Asn Gly Val Pro Ile
    290                 295                 300

Thr Gly Leu Thr Leu Ser Lys Ile Thr Gly Ser Val Ala Ser Ser Gly
305                 310                 315                 320

Thr Asn Val Tyr Ile Leu Cys Ala Ser Gly Ala Cys Ser Asn Trp Lys
                325                 330                 335

Trp Ser Gly Val Ser Val Thr Gly Gly Lys Lys Ser Thr Lys Cys Ser
            340                 345                 350

Asn Ile Pro Ser Gly Ser Gly Ala Ala Cys
        355                 360


<210> SEQ ID NO 28
<211> LENGTH: 333
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 28

Met Glu Thr Ser Lys Leu Phe Leu Leu Ser Ser Ser Leu Leu Leu Val
1               5                   10                  15

Leu Leu Ala Thr Arg Pro Cys Pro Ser Leu Ser Glu Arg Cys Asn Pro
            20                  25                  30

Lys Asp Lys Lys Val Leu Leu Gln Ile Lys Lys Ala Leu Asp Thr Pro
        35                  40                  45

Tyr Ile Leu Ala Ser Trp Asn Pro Asn Thr Asp Cys Cys Gly Trp Tyr
    50                  55                  60

Cys Val Glu Cys Asp Leu Thr Thr His Arg Ile Asn Ser Leu Thr Ile
65                  70                  75                  80

Phe Ser Gly Gln Leu Ser Gly Gln Ile Pro Asp Ala Val Gly Asp Leu
                85                  90                  95

Pro Phe Leu Glu Thr Leu Ile Phe Arg Lys Leu Ser Asn Leu Thr Gly
            100                 105                 110

Gln Ile Pro Pro Ala Ile Ala Lys Leu Lys His Leu Lys Met Val Arg
        115                 120                 125

Leu Ser Trp Thr Asn Leu Phe Gly Pro Val Pro Ala Phe Phe Ser Glu
    130                 135                 140

Leu Lys Asn Leu Thr Tyr Leu Asp Leu Ser Phe Asn Asn Leu Ser Gly
145                 150                 155                 160

Pro Ile Pro Gly Ser Leu Ser Leu Leu Pro Asn Leu Gly Ala Leu His
                165                 170                 175

Ile Asp Arg Asn His Leu Thr Gly Pro Ile Pro Asp Ser Phe Gly Lys
            180                 185                 190

Phe Ala Gly Ser Thr Pro Gly Leu His Leu Ser His Asn Gln Leu Ser
        195                 200                 205

Gly Lys Ile Pro Tyr Ser Phe Arg Gly Phe Asp Pro Asn Val Met Asp
    210                 215                 220

Leu Ser Arg Asn Lys Leu Glu Gly Asp Leu Ser Ile Phe Phe Asn Ala
225                 230                 235                 240

Asn Lys Ser Thr Gln Ile Val Asp Phe Ser Arg Asn Leu Phe Gln Phe
                245                 250                 255
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```
Asp Leu Ser Arg Val Glu Phe Pro Lys Ser Leu Thr Ser Leu Asp Leu
            260                 265                 270

Ser His Asn Lys Ile Ala Gly Ser Leu Pro Glu Met Met Thr Ser Leu
        275                 280                 285

Asp Leu Gln Phe Leu Asn Val Ser Tyr Asn Arg Leu Cys Gly Lys Ile
    290                 295                 300

Pro Val Gly Gly Lys Leu Gln Ser Phe Asp Tyr Asp Ser Tyr Phe His
305                 310                 315                 320

Asn Arg Cys Leu Cys Gly Ala Pro Leu Gln Ser Cys Lys
                325                 330


<210> SEQ ID NO 29
<211> LENGTH: 374
<212> TYPE: PRT
<213> ORGANISM: Botrytis cinerea

<400> SEQUENCE: 29

Met Val His Ile Thr Ser Leu Ile Ser Phe Leu Ala Ser Thr Ala Leu
1               5                   10                  15

Val Ser Ala Ala Pro Gly Ser Ala Pro Ala Asp Leu Asp Arg Arg Ala
            20                  25                  30

Gly Cys Thr Phe Ser Thr Ala Ala Thr Ala Ile Ala Ser Lys Thr Thr
        35                  40                  45

Cys Ser Thr Ile Ile Leu Asp Ser Val Val Val Pro Ala Gly Thr Thr
    50                  55                  60

Leu Asp Leu Thr Gly Leu Lys Thr Gly Thr Lys Val Ile Phe Gln Gly
65                  70                  75                  80

Thr Ala Thr Phe Gly Tyr Ser Glu Trp Glu Gly Pro Leu Ile Ser Ile
                85                  90                  95

Ser Gly Gln Asp Ile Val Val Thr Gly Ala Ser Gly Asn Lys Ile Asp
            100                 105                 110

Gly Gly Gly Ala Arg Trp Trp Asp Gly Leu Gly Ser Asn Val Ser Ala
        115                 120                 125

Gly Lys Gly Lys Val Lys Pro Lys Phe Phe Ser Ala His Lys Leu Thr
    130                 135                 140

Gly Ser Ser Ser Ile Thr Gly Leu Asn Phe Leu Asn Ala Pro Val Gln
145                 150                 155                 160

Cys Ile Ser Ile Gly Gln Ser Val Gly Leu Ser Leu Ile Asn Ile Asn
                165                 170                 175

Ile Asp Asn Ser Ala Gly Asp Ala Gly Asn Leu Gly His Asn Thr Asp
            180                 185                 190

Ala Phe Asp Ile Asn Leu Ser Gln Asn Ile Phe Ile Ser Gly Ala Ile
        195                 200                 205

Val Lys Asn Gln Asp Asp Cys Val Ala Val Asn Ser Gly Thr Asn Ile
    210                 215                 220

Thr Phe Thr Gly Gly Asn Cys Ser Gly Gly His Gly Leu Ser Ile Gly
225                 230                 235                 240

Ser Val Gly Gly Arg Ser Gly Thr Gly Ala Asn Asp Val Lys Asp Val
                245                 250                 255

Arg Phe Leu Ser Ser Thr Val Gln Lys Ser Thr Asn Gly Val Arg Val
            260                 265                 270

Lys Thr Val Ser Asp Thr Lys Gly Ser Val Thr Gly Val Thr Phe Gln
        275                 280                 285

Asp Ile Thr Leu Ile Gly Ile Thr Gly Val Gly Ile Asp Val Gln Gln
    290                 295                 300
```

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```
Asp Tyr Gln Asn Gly Ser Pro Thr Gly Thr Pro Thr Asn Gly Val Pro
305                 310                 315                 320

Ile Thr Gly Leu Thr Met Asn Asn Val His Gly Asn Val Ile Gly Gly
                325                 330                 335

Gln Asn Thr Tyr Ile Leu Cys Ala Asn Cys Ser Gly Trp Thr Trp Asn
            340                 345                 350

Lys Val Ala Val Thr Gly Gly Thr Val Lys Lys Ala Cys Ala Gly Val
        355                 360                 365

Pro Thr Gly Ala Ser Cys
    370
```

<210> SEQ ID NO 30
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 30

```
Met Asp Lys Thr Ala Thr Leu Cys Leu Leu Phe Leu Phe Thr Phe Leu
1               5                   10                  15

Thr Thr Cys Leu Ser Lys Asp Leu Cys Asn Gln Asn Asp Lys Asn Thr
            20                  25                  30

Leu Leu Lys Ile Lys Lys Ser Leu Asn Asn Pro Tyr His Leu Ala Ser
        35                  40                  45

Trp Asp Pro Gln Thr Asp Cys Cys Ser Trp Tyr Cys Leu Glu Cys Gly
    50                  55                  60

Asp Ala Thr Val Asn His Arg Val Thr Ala Leu Thr Ile Phe Ser Gly
65                  70                  75                  80

Gln Ile Ser Gly Gln Ile Pro Ala Glu Val Gly Asp Leu Pro Tyr Leu
                85                  90                  95

Glu Thr Leu Val Phe Arg Lys Leu Ser Asn Leu Thr Gly Thr Ile Gln
            100                 105                 110

Pro Thr Ile Ala Lys Leu Lys Asn Leu Arg Met Leu Arg Leu Ser Trp
        115                 120                 125

Thr Asn Leu Thr Gly Pro Ile Pro Asp Phe Ile Ser Gln Leu Lys Asn
    130                 135                 140

Leu Glu Phe Leu Glu Leu Ser Phe Asn Asp Leu Ser Gly Ser Ile Pro
145                 150                 155                 160

Ser Ser Leu Ser Thr Leu Pro Lys Ile Leu Ala Leu Glu Leu Ser Arg
                165                 170                 175

Asn Lys Leu Thr Gly Ser Ile Pro Glu Ser Phe Gly Ser Phe Pro Gly
            180                 185                 190

Thr Val Pro Asp Leu Arg Leu Ser His Asn Gln Leu Ser Gly Pro Ile
        195                 200                 205

Pro Lys Ser Leu Gly Asn Ile Asp Phe Asn Arg Ile Asp Leu Ser Arg
    210                 215                 220

Asn Lys Leu Gln Gly Asp Ala Ser Met Leu Phe Gly Ser Asn Lys Thr
225                 230                 235                 240

Thr Trp Ser Ile Asp Leu Ser Arg Asn Met Phe Gln Phe Asp Ile Ser
                245                 250                 255

Lys Val Asp Ile Pro Lys Thr Leu Gly Ile Leu Asp Leu Asn His Asn
            260                 265                 270

Gly Ile Thr Gly Asn Ile Pro Val Gln Trp Thr Glu Ala Pro Leu Gln
        275                 280                 285
```

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```
Phe Phe Asn Val Ser Tyr Asn Lys Leu Cys Gly His Ile Pro Thr Gly
    290                 295                 300

Gly Lys Leu Gln Thr Phe Asp Ser Tyr Ser Tyr Phe His Asn Lys Cys
305                 310                 315                 320

Leu Cys Gly Ala Pro Leu Glu Ile Cys Lys
                325                 330


<210> SEQ ID NO 31
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 31

Met Asp Lys Thr Met Thr Leu Phe Leu Leu Leu Ser Thr Leu Leu Leu
1               5                   10                  15

Thr Thr Ser Leu Ala Lys Asp Leu Cys His Lys Asp Asp Lys Thr Thr
            20                  25                  30

Leu Leu Lys Ile Lys Lys Ser Leu Asn Asn Pro Tyr His Leu Ala Ser
        35                  40                  45

Trp Asp Pro Lys Thr Asp Cys Cys Ser Trp Tyr Cys Leu Glu Cys Gly
    50                  55                  60

Asp Ala Thr Val Asn His Arg Val Thr Ser Leu Ile Ile Gln Asp Gly
65                  70                  75                  80

Glu Ile Ser Gly Gln Ile Pro Pro Glu Val Gly Asp Leu Pro Tyr Leu
                85                  90                  95

Thr Ser Leu Ile Phe Arg Lys Leu Thr Asn Leu Thr Gly His Ile Gln
            100                 105                 110

Pro Thr Ile Ala Lys Leu Lys Asn Leu Thr Phe Leu Arg Leu Ser Trp
        115                 120                 125

Thr Asn Leu Thr Gly Pro Val Pro Glu Phe Leu Ser Gln Leu Lys Asn
    130                 135                 140

Leu Glu Tyr Ile Asp Leu Ser Phe Asn Asp Leu Ser Gly Ser Ile Pro
145                 150                 155                 160

Ser Ser Leu Ser Ser Leu Arg Lys Leu Glu Tyr Leu Glu Leu Ser Arg
                165                 170                 175

Asn Lys Leu Thr Gly Pro Ile Pro Glu Ser Phe Gly Thr Phe Ser Gly
            180                 185                 190

Lys Val Pro Ser Leu Phe Leu Ser His Asn Gln Leu Ser Gly Thr Ile
        195                 200                 205

Pro Lys Ser Leu Gly Asn Pro Asp Phe Tyr Arg Ile Asp Leu Ser Arg
    210                 215                 220

Asn Lys Leu Gln Gly Asp Ala Ser Ile Leu Phe Gly Ala Lys Lys Thr
225                 230                 235                 240

Thr Trp Ile Val Asp Ile Ser Arg Asn Met Phe Gln Phe Asp Leu Ser
                245                 250                 255

Lys Val Lys Leu Ala Lys Thr Leu Asn Asn Leu Asp Met Asn His Asn
            260                 265                 270

Gly Ile Thr Gly Ser Ile Pro Ala Glu Trp Ser Lys Ala Tyr Phe Gln
        275                 280                 285

Leu Leu Asn Val Ser Tyr Asn Arg Leu Cys Gly Arg Ile Pro Lys Gly
    290                 295                 300
```

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```
Glu Tyr Ile Gln Arg Phe Asp Ser Tyr Ser Phe Phe His Asn Lys Cys
305                 310                 315                 320

Leu Cys Gly Ala Pro Leu Pro Ser Cys Lys
                325                 330
```

The invention claimed is:

1. A nucleic acid molecule coding for a chimeric protein comprising: an amino acid sequence with polygalacturonase inhibitor (PGIP) activity of plant origin; and an amino acid sequence with polygalacturonase (PG) activity of fungal, bacterial or insect origin;

wherein expression of the chimeric protein is under control of a non-constitutive promoter; and

wherein the amino acid sequence with the PGIP activity is separated from the amino acid sequence with the PG activity by a linker configured to prevent intramolecular interactions,

wherein the nucleic acid molecule comprises a nucleotide sequence consisting essentially of SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9.

2. The nucleic acid molecule according to claim **1**, wherein said chimeric protein comprises the amino acid sequence with the polygalacturonase inhibitor (PGIP) activity of plant origin at the N-terminal portion of the chimeric protein, and the amino acid sequence with the polygalacturonase (PG) activity of fungal, bacterial or insect origin at the C-terminal portion of the chimeric protein.

3. The nucleic acid molecule according to claim **1**, comprising a region coding for a signal peptide.

4. The nucleic acid molecule according to claim **1**, wherein the promoter regulates the expression of PR-1 gene of *Arabidopsis* (PPR-1).

5. The nucleic acid molecule of claim **1**, incorporated in an expression vector.

6. The expression vector according to claim **5**, wherein the nucleic acid molecule is under the control of the promoter which is active in plants.

7. The expression vector according to claim **6**, wherein the promoter is pathogen inducible.

8. A method for producing at least one transgenic plant cell, comprising: introducing the expression vector of claim **5** into at least one plant cell.

9. The nucleic acid molecule according to claim **1**, wherein the promoter is pathogen inducible.

10. The nucleic acid of claim **1** incorporated in at least one host cell.

11. The host cell of claim **10**, wherein the host cell is a plant cell.

* * * * *