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(54) PHYTOPHTHORA RESISTANT PLANTS BELONGING TO THE SOLANACEAE FAMILY

PHYTOPHTHORA-RESISTENTE PFLANZEN DER FAMILIE SOLANACEAE

PLANTES RÉSISTANTES À PHYTOPHTHORA APPARTENANT À LA FAMILLE DES SOLANACÉES

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Description

[0001] The present invention relates to *Phytophthora* resistance plants belonging to *Solanaceae* family wherein said resistance is encoded by a combination of two genes.

[0002] The plant pathogen *Phytophthora* is a genus of plant-damaging Oomycetes (water molds), whose member species are capable of causing large economic losses on crops worldwide, as well as environmental damage in natural ecosystems. The genus was first described by Heinrich Anton de Bary in 1875. Approximately 100 species have been described, although and estimate of 100 to 500 undiscovered *Phytophthora* species are suspected to exist.

[0003] *Phytophthora* pathogens are mostly pathogens of dicotyledons and generally are host-specific parasites. Many species of *Phytophthora* are plant pathogens of considerable economic importance. *Phytophthora infestans* was the infective agent of the potato blight that caused the Great Irish Famine (1845-1849), and still remains the most destructive pathogen of solanaceous crops, including tomato and potato. The soya bean root and stem rot agent, *Phytophthora sojae,* has also caused longstanding problems for the agricultural industry. In general, plant diseases caused by this genus are difficult to control chemically, and thus the growth of resistant cultivars is the main management strategy. Other important Phytophthora diseases are: *Phytophthora cactorum* - causes rhododendron root rot affecting rhododendrons, azaleas and causes bleeding canker in hardwood trees; *Phytophthora capsici* - infects Cucurbitaceae fruits, such as cucumbers and squash, *Phytophthora cinnamomi* - causes cinnamon root rot affecting woody ornamentals including arborvitae, azalea, *Phytophthora fragariae* - causes red root rot affecting strawberries; *Phytophthora kernoviae* - pathogen of beech and rhododendron, also occurring on other trees and shrubs including oak, and holm oak, *Phytophthora megakarya* - one of the cocoa black pod disease species, is invasive and probably responsible for the greatest cocoa crop loss in Africa; *Phytophthora palmivora* - causes fruit rot in coconuts and betel nuts, *Phytophthora ramorum*, *Phytophthora quercina* - causes oak death, and *Phytophthora sojae* - causes soybean root rot.

[0004] *Phytophthora* is sometimes referred to as a fungal-like organism but it is classified under a different kingdom: *Chromalveolata* (formerly *Stramenopila* and previously *Chromista*). *Phytophthora* is morphologically very similar to true fungi yet its evolutionary history is quite distinct. In contrast to fungi, chromalveolatas are more closely related to plants than animals. Whereas fungal cell walls are made primarily of chitin, chromalveolata cell walls are constructed mostly of cellulose. Ploidy levels are different between these two groups; *Phytophthora* have diploid (paired) chromosomes in the vegetative (growing, non-reproductive) stage of life, Fungi are almost always haploid in this state. Biochemical pathways also differ, notably the highly conserved.

[0005] *Phytophthoras* may reproduce sexually or asexually. In many species, sexual structures have never been observed, or have only been observed in laboratory matings. In homothallic species, sexual structures occur in single culture. Heterothallic species have mating strains, designated as A1 and A2. When mated, antheridia introduce gametes into oogonia, either by the oogonium passing through the antheridium (amphigyny) or by the antheridium attaching to the proximal (lower) half of the oogonium (paragyny), and the union producing oospores. Like animals, but not like most true fungi, meiosis is gametic, and somatic nuclei are diploid. Asexual (mitotic) spore types are chlamydospores, and sporangia which produce zoospores. Chlamydospores are usually spherical and pigmented, and may have a thickened cell wall to aid in its role as a survival structure. Sporangia may be retained by the subtending hyphae (non-caducous) or be shed readily by wind or water tension (caducous) acting as dispersal structures. Also, sporangia may release zoospores, which have two unlike flagella which they use to swim towards a host plant.

[0006] *The Solanaceae,* or nightshades, are an economically important family of flowering plants. The family ranges from herbs to trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. Many members of the family contain potent alkaloids, and some are highly toxic.

[0007] The family belongs to the order *Solanales,* in the asterid group dicotyledons (*Magnoliopsida*). The solanaceae family consists of approximately 98 genera and some 2,700 species, with a great diversity of habitats, morphology and ecology.

[0008] The family has a worldwide distribution being present on all continents except Antarctica. The greatest diversity in species is found in South America and Central America. *Solanaceae* includes a number of commonly collected or cultivated species. Perhaps the most economically important genus of the family is *Solanum,* which contains the potato (*Solanum tuberosum,* in fact, another common name of the family is the "potato family"), the tomato (*Solanum lycopersicum*), and the aubergine or eggplant (*Solanum melongena*). Another important genus *Capsicum* produce both chilli peppers and bell peppers.

[0009] The genus *Physalis* produces the so-called groundcherries, as well as the tomatillo (*Physalis philadelphica*), the Cape gooseberry and the Chinese lantern. The genus *Lycium* contains the boxthorns and the wolfberry *Lycium barbarum. Nicotiana* contains, among other species, the plant that produces tobacco. Some other important members of *Solanaceae* include a number of ornamental plants such as Petunia, Browallia and Lycianthes, the source of psychoactive alkaloids, Datura, Mandragora (mandrake), and Atropa belladonna (deadly nightshade). Certain species are universally known for their medicinal uses, their psychotropic effects or for being poisonous.

[0010] With the exception of tobacco (*Nicotianoideae*) and petunia (*Petunioideae*), most of the economically important

genera are contained in the subfamily *Solanoideae*. Finally, but not less importantly, the solanaceas include many model organisms which are important in the investigation of fundamental biological questions at a cellular, molecular and genetic level, such as tobacco and the petunia.

[0011] WO 2008/092505 discloses plants being resistant to a pathogen of viral, bacterial, fungal or oomycete origin, wherein the plants have a reduced level, reduced activity or complete absence of DMR6 protein as compared to a plant that is not resistant to the pathogen, in particular organisms of the Fungi or the phylum Oomycota.

[0012] Considering the economic importance of many plant members of the *Solanaceae* family and the destructive effect of the plant pathogen *Phytophthora* on many members of this family, it is an object, amongst other objects, of the present invention to provide *Phytophthora* resistant plants.

[0013] The above object, amongst other objects, is met by the present invention by providing plants as outlined in the appended claims.

[0014] Specifically, the above object, amongst other objects, is met, according to a first aspect, by plants belonging to the *Solanaceae* family wherein the present plants comprise a genetic trait providing *Phytophthora* resistance and wherein the present resistance trait is encoded by a combination of at least two genes having a reduced expression, or reduced transcription, of the present genes or a reduced activity of proteins encoded by the present genes as compared to the plant belonging to *Solanaceae* family being susceptible to *Phytophthora*. According to the present invention, the present plants belonging to the *Solanaceae* family are selected from the group consisting of potato, petunia and tomato.

[0015] The present invention relates to potato, the present *Phytophthora* resistance is resistance to *Phytophthora infestans* and the present combination of at least two genes are genes encoding proteins according to SEQ ID No. 1 and SEQ ID No. 2 or proteins having at least 90% sequence identity with SEQ ID No. 1 and SEQ ID No. 2, such as 91%, 92%, 93% and 94% sequence identity, preferably at least 95% sequence identity, such as 96%, 97%, 98% and 99% sequence identity.

[0016] The present invention relates to petunia, the present *Phytophthora* resistance is resistance to *Phytophthora nicotianae* and the present combination of at least two genes are genes encoding proteins according to SEQ ID No. 3 and SEQ ID No. 4 or proteins having at least 90% sequence identity with SEQ ID No. 3 and SEQ ID No. 4, such as 91%, 92%, 93% and 94% sequence identity, preferably at least 95% sequence identity, such as 96%, 97%, 98% and 99% sequence identity.

[0017] The present invention relates to tomato, the present *Phytophthora* resistance is resistance to *Phytophthora infestans* and the present combination of at least two genes are genes encoding proteins according to SEQ ID No. 5 and SEQ ID No. 6 or proteins having at least 90% sequence identity with SEQ ID No. 5 and SEQ ID No. 6, such as 91%, 92%, 93% and 94% sequence identity, preferably at least 95% sequence identity, such as 96%, 97%, 98% and 99% sequence identity.

[0018] The present invention relates to a plant belonging to the *Solanaceae* family wherein the present plant comprises a genetic trait providing *Phytophthora* resistance, wherein the present resistance trait is obtained by down regulating the activity of combination of two genes or reducing the activity of proteins encoded by the present genes in a *Phytophthora* susceptible plant, wherein the present two genes encode the combinations of SEQ ID Nos. 1 and 2 or SEQ ID Nos. 3 and 4 or SEQ ID Nos. 5 and 6 or proteins having at least 90% sequence identity therewith such as 91%, 92%, 93% and 94% sequence identity, preferably at least 95% sequence identity, such as 96%, 97%, 98% and 99% sequence identity.

[0019] Given the advantageous properties of the disclosed genes for providing *Phytophthora* resistance plants, discloses is the use of genes encoding the combinations of SEQ ID Nos. 1 and 2 or SEQ ID Nos. 3 and 4 or SEQ ID Nos. 5 and 6 or proteins having at least 90% sequence identity therewith, such as 91%, 92%, 93% and 94% sequence identity, preferably at least 95% sequence identity, such as 96%, 97%, 98% and 99% sequence identity, for providing *Phytophthora* resistance in plants belonging to the *Solanaceae* family.

[0020] The disclosed use for providing *Phytophthora* resistance in plants belonging to the *Solanaceae* family comprises reduced expression of the present genes or a reduced activity of proteins encoded by the present genes as compared to the plant belonging to *Solanaceae* family being susceptible to *Phytophthora*.

[0021] The present plants belonging to the *Solanaceae* family are selected from the group consisting of potato, petunia and tomato. The present *Phytophthora* resistance is *Phytophthora infestans* in potato and/or tomato, or *Phytophthora nicotianae* in petunia.

[0022] Given the *Phytophthora* resistance providing properties of the present proteins and genes, disclosed are proteins and genes suitable for providing *Phytophthora* resistance to plants. Specifically disclosed are proteins selected from the group consisting of SEQ ID No. 1, 2, 3, 4, 5, 6.

[0023] Disclosed are coding sequences, or genes encoding cDNA sequence, selected from the group consisting of SEQ ID No. 7, 8, 9, 10, 11, 12.

[0024] The invention is further illustrated in the examples below, with reference to the figures, wherein:

Figure 1 shows a detached leaf assay of control potato plants after infection with *Phytophthora infestans,* wherein all leaves are infected by *Phytophthora infestans*.

Figure 2 shows a detached leaf assay of SEQ ID NOS. 7 & 8 silenced potato plants after infection with *Phytophthora infestans,* wherein each leaf is from an independent plant. Figure 2a shows leaves from plants silenced with a middle construct, silencing both SEQ ID NOS. 7&8. Figure 2b shows leaves from chimeric silenced plants.

Figure 3 shows the percentage of plants which are infected by *Phytophthora infestans,* wherein the first bar shows a control group (about 10% is partially)infected, the second bar shows plants of which only SEQ ID NO 7 is silenced (about 10% partially infected), the third bar shows plants of which both SEQ ID NO 7 and 8 is silenced in the middle part of the respective sequences (about 50% clean), the fourth bar shows plants of which both SEQ ID NO 7 and 8 is silenced at the 5'end (about 40% clean).

Figure 4 shows the percentages of living petunia plants after inoculation with *Phytophthora nicotianae,* wherein the first bar shows wild type control plant (0% living), the second bar shows SEQ ID NO 9 mutants (20% living plants), the third bar shows SEQ ID NO 10 mutants (20% living plants) and the fourth bar shows double mutants, i.e. both SEQ ID NO 9 and 10 (45% living plants).

Figure 5 shows leaves of tomato plants from a *Phytophthora infestans* disease test.

Examples

Example 1 (potato)

RNAi constructs targeting potato SEQ ID NOS. 7 and 8

[0025] 3 different RNAi constructs were made, harboring/targeting:

1. 5' end of SEQ ID NO 7: equivalent to coding sequence -159-200 (-159 from start means in 5'utr).
2. Chimera of 5' end of SEQ ID NOS. 7 and 8: equivalent to coding sequence 4-199 + 1-204.
3. Middle part of SEQ ID NO. 7 (highly homologous to middle of SEQ ID NO 8): equivalent to coding sequence 334-743.

[0026] The fragments were amplified from genomic DNA and cloned into the pENTR-D-TOPO vector. For the chimeric construct, 2 fragments were coupled using primers with complementary overhangs, and subsequent extension and amplification to create the fused fragment. Fragments were transferred using a Gateway LR reaction to the RNAi vector pK7GWiWG2 (Karimi et al., 2002, Trends Plant Sci 7), creating an inverted repeat with hairpin structure. Because the pK7GWiWG2 vector requires Streptomycin for bacterial selection, and the Agrobacterium strain used for potato transformation (LBA4404) already carries a Streptomycin selection marker, the complete RNAi (hairpin) cassette was transferred to a different plant transformation vector, pGreen0029 (bacterial as well as plant selection marker = Kanamycin) (Hellens et al., 2000, Plant Mol Biol 42). The final constructs allow stable expression of a 35S-promoter driven hairpin RNA that forms a silencing-inducing dsRNA, after the hairpin-loop forming intron gets spliced out. At least six independent T1 transformants were maintained for each construct.

Phytophthora infestans Assay details

[0027] Detached leaves were taken from T1 (first generation transgenics) plants, and placed in a tray with 100% RH with petioles in wet cotton-wool or Oasis. *Phytophthora infestans* (P.inf) zoospores/sporangia were harvested from P.inf cultures (rye-sucrose-agar plates), and a 10ul drop of spore suspension containing 10e3 sporangia (10e5/ml) was placed on each side of the midvein. Trays were incubated at 18C. Leaf infection rates were scored on day 11, as 1. Completely infected/overgrown, 2. Partially infected (10-50% area), and 3. Clean (<10% area).

[0028] As shown in figures 1 and 2, the double silenced (SEQ ID NO. 7 & 8) plants of figure 2a show that only 50% is infected, the double silenced (chimeric) plants of figure 2b show that only 60% is infected, whereas the control group of figure 1 shows that all plants were infected. As shown in figure 3, 40 to 50% of the both SEQ ID NO. 7 and SEQ ID NO. 8 silenced plants are clean, whereas the plants having only SEQ ID NO. 7 silenced only 10% of the plants score partially infected. Accordingly, silencing of both SEQ ID NO. 7 and 8 provides resistance to *Phytophthora infestans.*

Example 2 (petunia)

[0029] Transposon insertion lines were identified from a collection/library (Vandenbussche et al., 2008, Plant Journal 54). 2 dTph1 transposon insertion alleles were found in SEQ ID NO 9 and 3 dTph1 transposon insertion alleles in SEQ ID NO 10. Several crosses were made to generate double mutants.

***Phytophthora nicotianae* Assay details**

[0030] Plants were grown in standard potting soil, individually potted, at 23C.
P.nicotianae spores were harvested from cultures (lima-bean-agar or V8-agar plates), and 2ml of spore suspension containing 10e4 (assay Sept) spores was dripped onto the soil with each plant. Plant collapse was monitored regularly.
[0031] As shown in figure 4, double mutants, i.e. plants having mutations in both SEQ ID NO 9 and SEQ ID NO 10 have a percentage of living plants of 45%, whereas the percentage of living plants of single mutants (mutant in SEQ ID NO. 9 or SEQ ID NO. 10) is only 20%.

Example 3 (tomato)

[0032] Tomato plants were transformed with two constructs, either for providing over expression of both SEQ ID NO. 11 and 12, or for providing silencing of both SEQ ID NO. 11 and 12.
[0033] Tomato SEQ ID NO. 11 silencing constructs were generated using Gateway cloning of a 300 bp fragment identical to the middle part of the CDS of SEQ ID NO. 11.

Sequence:

[0034]

TTGGGTGAACAAGGACAACATATGGCTATCAATTATTATCCTCCTTGTCCACAACCAG
AACTTACTTATGGGCTTCCGGCCCATACTGATCCAAATTCACTTACAATTCTTCTTCAA
GACTTGCAAGTTGCGGGTCTTCAAGTTCTTAAAGATGGCAAATGGTTAGCTGTAAAAC
CTCAACCTGACGCCTTTGTCATTAATCTTGGGGATCAATTGCAGGCAGTAAGTAACGG
TAAGTACAGAAGTGTATGGCATCGAGCTATTGTGAATTCAGATCAAGCTAGGATGTCA
GTGGCTTCGTTT

Using primers:

[0035]

S. Lycopersicum AttB1-F	aaaaagcaggcttcttgggtgaacaaggacaaca
S.Lycopersicum AttB2-R	agaaagctgggtaaaacgaagccactgacatcc

[0036] The generated ENTRY vector was Gateway cloned into the pHellsgate12 binary vector. Following Agrobacterium transformation according standard procedure for tomato. The silencing constructs were able to silence both SEQ ID NO. 11 and 12, due to similarities in the sequences.
[0037] Offspring from transformed tomato plants were subjected to a disease test by inoculation of *Phytophthora infestans* isolate US11. 7 days after inoculation the plants were visually analysed by scoring leaves on a visual scale from 1 to 9, wherein 1 means susceptible and 9 means resistant. As a control for susceptible the plants TS33, TS19 and OT9 were used. As control for resistant the known resistant wild accession LA1269 is used. Per plant 8 leaves were measured. Table below provides the average score from the 8 leaves per plant.

LA1269	RC	8,7
TS33	VC	1,3
TS19	VC	1,5
OT9	VC	2,0
551-06-01	overexpres	2,8
551-06-02	overexpres	3,3
551-06-03	overexpres	3,0
551-06-07	overexpres	1,5
551-06-08	overexpres	2,3
551-06-09	overexpres	2,3
551-06-12	overexpres	2,3

556-02-01	silencing	6,5
556-02-02	silencing	8,5
556-02-03	silencing	8,3
556-02-06	silencing	7,3
556-02-11	silencing	7,3
556-01-01	silencing	7,8
556-01-02	silencing	8,3
556-01-03	silencing	8,5
556-01-04	silencing	8,5
556-01-05	silencing	8,5
556-01-06	silencing	6,0
556-01-07	silencing	5,5
556-01-08	silencing	8,5
556-01-09	silencing	7,0
556-01-10	silencing	8,5
556-01-11	Silencing	8,8
556-01-12	Silencing	7,8

[0038] In the table is shown that the SEQ ID NO. 11 and 12overexpressing plants are susceptible for isolate US11. The silenced plant provides significant higher scores than the susceptible control LA1269.. For example plant 556-01-08 has an average score of 8.5. A sample of this plant is shown in figure 5 in box G10, and is not infected similar to resistant control plant LA1296 as shown in box D8. Accordingly, silencing of both SEQ ID NO. 11 and 12 provides resistance to *Phytophthora infestans.*

SEQUENCE LISTING

[0039]

<110> Enza Zaden Beheer B.V.

<120> PHYTOPHTHORA REASISTANT PLANTS BELONGING TO THE SOLANACEAE FAMILY

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<160> 12

<170> BiSSAP 1.2

<210> 1
<211> 337
<212> PRT
<213> Solanum tuberosum

<400> 1

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Gln Ser Tyr Ile Arg Pro Glu Ser Asp Arg Pro Arg Leu Ser Asp Val
            20                  25                  30
Val Asp Cys Glu Asn Val Pro Ile Ile Asp Leu Gly Cys Gly Asp Gln
        35                  40                  45
Ala Gln Ile Ile Arg Leu Ile Gly Glu Ala Cys Gln Thr Tyr Gly Phe
    50                  55                  60
Phe Gln Val Ile Asn His Gly Val Pro Lys Glu Val Val Glu Lys Met
65                  70                  75                  80
Leu Gly Val Ala Gly Glu Phe Phe Asn Leu Pro Val Glu Glu Lys Leu
                85                  90                  95
Lys Leu Tyr Ser Asp Asp Pro Ser Lys Thr Met Arg Leu Ser Thr Ser
            100                 105                 110
Phe Asn Val Lys Lys Glu Thr Val His Asn Trp Arg Asp Tyr Leu Arg
        115                 120                 125
Leu His Cys His Pro Leu Glu Lys Tyr Ala Pro Glu Trp Pro Ser Asn
    130                 135                 140
Pro Ser Ser Phe Arg Asp Ile Val Ser Arg Tyr Cys Thr Glu Val Arg
145                 150                 155                 160
Gln Leu Gly Phe Arg Leu Glu Glu Ala Ile Ala Glu Ser Leu Gly Leu
                165                 170                 175
Glu Lys Glu Cys Ile Lys Asp Val Leu Gly Glu Gln Gly Gln His Met
            180                 185                 190
Ala Ile Asn Phe Tyr Pro Pro Cys Pro Gln Pro Glu Leu Thr Tyr Gly
        195                 200                 205
Leu Pro Ala His Thr Asp Pro Asn Ser Leu Thr Ile Leu Leu Gln Asp
    210                 215                 220
Leu Gln Val Ser Gly Leu Gln Val Leu Lys Asp Gly Lys Trp Leu Ala
225                 230                 235                 240
Val Lys Pro Gln Pro Asp Ala Phe Val Ile Asn Leu Gly Asp Gln Leu
                245                 250                 255
Gln Ala Val Ser Asn Gly Lys Tyr Lys Ser Val Trp His Arg Ala Ile
            260                 265                 270
Val Asn Ser Asp Gln Ala Arg Met Ser Val Ala Ser Phe Leu Cys Pro
        275                 280                 285
Cys Asp Ser Ala Lys Ile Ser Ala Pro Lys Leu Leu Thr Glu Asp Gly
    290                 295                 300
Ser Pro Val Ile Tyr Gln Asp Phe Thr Tyr Ala Glu Tyr Tyr Lys Lys
305                 310                 315                 320
Phe Trp Ser Arg Asn Leu Asp Gln Glu His Cys Leu Glu Leu Phe Lys
                325                 330                 335
Asn
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<400> 2

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            20                  25                  30
Val Val Asp Arg Asp Asp Leu Val Pro Val Ile Asp Met Ser Cys Thr
        35                  40                  45
Asp Arg Asn Val Ile Val His Gln Ile Gly Glu Ala Cys Arg Leu Tyr
    50                  55                  60
Gly Phe Phe Gln Val Ile Asn His Gly Val Ser Lys Lys Val Met Asp
65                  70                  75                  80
Glu Met Leu Gly Val Ala His Glu Phe Phe Lys Leu Pro Val Glu Glu
                85                  90                  95
Lys Met Lys Leu Tyr Ser Asp Asp Pro Ser Lys Thr Met Arg Leu Ser
            100                 105                 110
Thr Ser Phe Asn Val Lys Lys Glu Thr Val His Asn Trp Arg Asp Tyr
        115                 120                 125
Leu Arg Leu His Cys Tyr Pro Leu Asp Lys Tyr Ala Pro Glu Trp Pro
    130                 135                 140
Ser Asn Pro Pro Ser Phe Arg Glu Ile Val Ser Lys Tyr Cys Met Glu
145                 150                 155                 160
Val Arg Gln Val Gly Tyr Arg Leu Glu Glu Ala Ile Ser Glu Ser Leu
                165                 170                 175
Gly Leu Glu Lys Asp Cys Ile Lys Asn Val Leu Gly Glu Gln Gly Gln
            180                 185                 190
His Met Ala Ile Asn Phe Tyr Pro Pro Cys Pro Gln Pro Glu Leu Thr
        195                 200                 205
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                245                 250                 255
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        275                 280                 285
Cys Pro Asn Asp Cys Ser Ile Ile Ser Ala Pro Lys Thr Leu Ile Glu
    290                 295                 300
Asp Gly Ser Ser Ala Ile Tyr Arg Asp Phe Thr Tyr Thr Glu Tyr Tyr
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Phe Lys Asn Asp Gly Thr
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<212> PRT
<213> Petunia

<400> 3

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Lys Ser Tyr Val Arg Pro Glu Ser Gln Arg Pro Arg Leu Ser Glu Val
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            35                  40                  45
Asn Asn Val Ile Ile Gln Gln Ile Glu Glu Ala Cys Arg Leu Tyr Gly
        50                  55                  60
Phe Phe Gln Val Ile Asn His Gly Val Pro Lys Lys Leu Ile Glu Glu
65                  70                  75                  80
Met Leu Gly Val Ala His Glu Phe Phe Lys Leu Pro Val Glu Glu Lys
                85                  90                  95
Met Lys Leu Tyr Ser Asp Asp Pro Ser Lys Thr Met Arg Leu Ser Thr
            100                 105                 110
Ser Phe Asn Val Lys Lys Glu Thr Val His Asn Trp Arg Asp Tyr Leu
        115                 120                 125
Arg Leu His Cys Tyr Pro Leu Glu Lys Tyr Ala Pro Glu Trp Pro Ser
    130                 135                 140
Thr Pro Ser Ser Phe Arg Glu Ile Val Ser Arg Tyr Cys Ile Glu Val
145                 150                 155                 160
Arg Gln Leu Gly Tyr Arg Leu Gln Glu Ala Ile Ser Glu Ser Leu Gly
                165                 170                 175
Leu Glu Lys Asp Cys Ile Lys Asn Ile Leu Gly Glu Gln Gly Gln His
            180                 185                 190
Met Ala Val Asn Tyr Tyr Pro Pro Cys Pro Glu Pro Glu Leu Thr Tyr
        195                 200                 205
Gly Leu Pro Ala His Thr Asp Pro Asn Ala Leu Thr Ile Leu Leu Gln
    210                 215                 220
Asp Leu Gln Val Ala Gly Leu Gln Val Leu Lys Asp Gly Lys Trp Leu
225                 230                 235                 240
Ser Val Lys Pro Arg Ala Asn Ala Phe Val Ile Asn Leu Gly Asp Gln
                245                 250                 255
Leu Gln Ala Leu Ser Asn Gly Lys Tyr Arg Ser Val Trp His Arg Ala
            260                 265                 270
Ile Val Asn Ser Asp Lys Pro Arg Leu Ser Val Ala Ser Phe Leu Cys
        275                 280                 285
Pro Ser Asp Cys Ala Ile Ile Ser Ala Pro Lys Thr Leu Thr Glu Asp
    290                 295                 300
Gly Ser Pro Thr Ile Tyr Arg Asp Phe Thr Tyr Pro Glu Tyr Tyr Lys
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Thr Leu Ile Ile His Gln Ile Ser Glu Ala Cys Arg Leu Tyr Gly Phe
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Phe Gln Val Ile Asn His Gly Val Pro Lys Lys Ile Val Glu Glu Met
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Leu Glu Ile Ala Gly Glu Phe Phe Arg Leu Pro Val Glu Glu Lys Leu
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Lys Leu Tyr Ser Asp Asp Pro Ser Lys Thr Met Arg Leu Ser Thr Ser
            100                 105                 110

Phe Asn Val Lys Lys Glu Thr Val His Asn Trp Arg Asp Tyr Leu Arg
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Leu His Cys Tyr Pro Leu Glu Lys Tyr Ala Pro Glu Trp Pro Ser Asn
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Pro Ser Ser Phe Arg Glu Ile Val Ser Arg Tyr Cys Thr Glu Val Arg
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Gln Leu Gly Phe Arg Leu Gln Glu Ala Ile Ala Glu Ser Leu Gly Leu
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Glu Lys Glu Cys Ile Lys Asp Val Leu Gly Glu Gln Gly Gln His Met
            180                 185                 190
Ala Ile Asn Phe Tyr Pro Pro Cys Pro Glu Pro Glu Leu Thr Tyr Gly
        195                 200                 205
Leu Pro Ala His Thr Asp Pro Asn Ala Leu Thr Ile Leu Leu Gln Asp
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225                 230                 235                 240
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Gln Ala Val Ser Asn Gly Arg Tyr Lys Ser Val Trp His Arg Ala Val
            260                 265                 270
Val Asn Thr Glu Asn Ala Arg Met Ser Val Ala Ser Phe Leu Cys Pro
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Cys Asp Ser Ala Lys Ile Ser Ala Pro Lys Leu Leu Thr Asp Asp Gly
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Ser Pro Ile Ile Tyr Arg Asp Phe Thr Tyr Ala Glu Tyr Tyr Lys Lys
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Ala Gln Ile Ile Arg Gln Ile Gly Glu Ala Cys Gln Thr Tyr Gly Phe
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Phe Gln Val Ile Asn His Gly Val Pro Lys Glu Val Val Glu Lys Met
65                  70                  75                  80
Leu Gly Val Ala Gly Glu Phe Phe Asn Leu Pro Val Glu Glu Lys Leu
                85                  90                  95
Lys Leu Tyr Ser Asp Asp Pro Ser Lys Thr Met Arg Leu Ser Thr Ser
            100                 105                 110
Phe Asn Val Lys Lys Glu Thr Val His Asn Trp Arg Asp Tyr Leu Arg
        115                 120                 125
Leu His Cys Tyr Pro Leu Glu Lys Tyr Ala Pro Glu Trp Pro Ser Asn
    130                 135                 140
Pro Ser Ser Phe Arg Glu Ile Val Ser Arg Tyr Cys Arg Glu Ile Arg
145                 150                 155                 160
Gln Leu Gly Phe Arg Leu Glu Glu Ala Ile Ala Glu Ser Leu Gly Leu
                165                 170                 175
Asp Lys Glu Cys Ile Lys Asp Val Leu Gly Glu Gln Gly Gln His Met
            180                 185                 190
Ala Ile Asn Tyr Tyr Pro Pro Cys Pro Gln Pro Glu Leu Thr Tyr Gly
        195                 200                 205
Leu Pro Ala His Thr Asp Pro Asn Ser Leu Thr Ile Leu Leu Gln Asp
    210                 215                 220
Leu Gln Val Ala Gly Leu Gln Val Leu Lys Asp Gly Lys Trp Leu Ala
225                 230                 235                 240
Val Lys Pro Gln Pro Asp Ala Phe Val Ile Asn Leu Gly Asp Gln Leu
                245                 250                 255
Gln Ala Val Ser Asn Gly Lys Tyr Arg Ser Val Trp His Arg Ala Ile
            260                 265                 270
Val Asn Ser Asp Gln Ala Arg Met Ser Val Ala Ser Phe Leu Cys Pro
        275                 280                 285
Cys Asp Ser Ala Lys Ile Ser Ala Pro Lys Leu Leu Thr Glu Asp Gly
    290                 295                 300
Ser Pro Val Ile Tyr Gln Asp Phe Thr Tyr Ala Glu Tyr Tyr Asn Lys
305                 310                 315                 320
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Glu Val Val Asp Ser Asp Asp Leu Val Pro Val Ile Asp Met Ser Cys
        35                  40                  45
Thr Asp Arg Asn Val Ile Val His Gln Ile Gly Glu Ala Cys Arg Leu
    50                  55                  60
Tyr Gly Phe Phe Gln Val Ile Asn His Gly Val Ser Lys Lys Ala Met
65                  70                  75                  80
Asp Glu Met Leu Gly Thr Met Arg Leu Ser Thr Ser Phe Asn Val Lys
                85                  90                  95
Lys Glu Thr Val His Asn Trp Arg Asp Tyr Leu Arg Leu His Cys Tyr
            100                 105                 110
Pro Leu Asp Lys Tyr Ala Pro Glu Trp Pro Ser Asn Pro Pro Ser Phe
        115                 120                 125
Arg Glu Ile Val Ser Lys Tyr Cys Met Glu Val Arg Glu Leu Gly Tyr
    130                 135                 140
Arg Leu Glu Glu Ala Ile Ser Glu Ser Leu Gly Leu Glu Lys Asp Cys
145                 150                 155                 160
Ile Lys Asn Val Leu Gly Glu Gln Gly Gln His Met Ala Ile Asn Phe
                165                 170                 175
Tyr Pro Gln Cys Pro Gln Pro Glu Leu Thr Tyr Gly Leu Pro Ala His
            180                 185                 190
Thr Asp Pro Asn Ala Ile Thr Ile Leu Leu Gln Asp Leu Gln Val Ala
        195                 200                 205
Gly Leu Gln Val Leu Lys Asp Gly Lys Trp Leu Ser Ile Lys Pro Gln
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Pro Asn Ala Phe Val Ile Asn Leu Gly Asp Gln Leu Glu Ala Leu Ser
225                 230                 235                 240
Asn Gly Lys Tyr Lys Ser Ile Trp His Arg Ala Ile Val Asn Ser Asp
                245                 250                 255
Lys Ala Arg Met Ser Val Ala Ser Phe Leu Cys Pro Asn Asp Cys Ser
            260                 265                 270
Ile Ile Ser Ala Pro Lys Thr Leu Thr Glu Asp Gly Ser Ser Ala Ile
        275                 280                 285

Tyr Arg Asp Phe Thr Tyr Ala Glu Tyr Tyr Glu Lys Phe Trp Ser Arg
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Asn Leu Asp Gln Glu Tyr Cys Leu Glu Leu Phe Lys Asn Asp Gly Thr
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ctaggggtag ctggggaatt tttcaatcta ccagtagaag agaagctaaa attgtattca     300
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cataattgga gagattatct cagacttcat tgtcatcctc tggagaaata tgctcctgaa     420
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cctgaatggc cttctaatcc tccttctttc agggaaatag tgagcaaata ttgcatggaa 480

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cgtctttatg gctttttca ggtaataaac catggtgtac caaagaaact aatagaggaa 240

atgctagggg tagctcatga gtttttcaag ctaccagtgg aagagaagat gaagttgtac 300

tcagatgatc catcaaagac catgagatta tcaacaagtt ttaatgtgaa gaaggaaact 360

gttcataatt ggagagacta tcttagattg cactgctatc ctttggagaa atatgcccct 420

gaatggcctt ctactccctc ttctttcagg gaaatcgtta gcagatattg catagaagtt 480

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ctgtcagtgg cttctttctt gtgtcctagt gattgtgcga taatcagtgc tccaaaaacc 900

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gatgatcctt caaagaccat gagattatca acaagtttta atgttaaaaa ggagacagtt 360

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tggccttcta atccatcatc tttcagggaa atcgtgagca gatattgcag ggaaattcgt 480

caactcggat ttagattaga agaagccata gcagaaagcc tggggttaga taaagagtgt 540

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gcttgtcgtc tttatgggtt ttttcaggtg ataaatcacg gtgtgtcgaa gaaggcgatg     240

gatgaaatgt tagggactat gagattatca actagtttta atgttaagaa ggaaactgtt     300

cataattgga gagattatct taggctacat tgttatcctt tggacaaata tgcccctgaa     360

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tag                                                                  963
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Claims

1. Plant belonging to the *Solanaceae* family wherein said plant comprises a genetic trait providing *Phytophthora* resistance and wherein said resistance trait is encoded by a combination of at least two genes having a reduced expression of said genes as compared to said plant belonging to *Solanaceae* family being susceptible to *Phytophthora* wherein:

   - said plant is potato, said *Phytophthora* resistance is resistance to *Phytophthora infestans* and said combination of at least two genes are genes encoding proteins according to SEQ ID No. 1 and SEQ ID No. 2 or proteins having at least 90% sequence identity with SEQ ID No. 1 and SEQ ID No. 2; or
   - said plant is petunia, said *Phytophthora* resistance is resistance to *Phytophthora nicotianae* and said combination of at least two genes are genes encoding proteins according to SEQ ID No. 3 and SEQ ID No. 4 or proteins having at least 90% sequence identity with SEQ ID No. 3 and SEQ ID No. 4; or
   - said plant is tomato, said *Phytophthora* resistance is resistance to *Phytophthora infestans* and said combination of at least two genes are genes encoding proteins according to SEQ ID No. 5 and SEQ ID No. 6 or proteins having at least 90% sequence identity with SEQ ID No. 5 and SEQ ID No. 6.

Patentansprüche

1. Zur Familie der *Solanaceae* gehörige Pflanze, wobei diese Pflanze ein genetisches Merkmal aufweist, das *Phytophthora-Resistenz* verleiht, und wobei dieses Resistenzmerkmal durch eine Kombination von wenigstens zwei Genen codiert ist, die im Vergleich zu der zur Familie der *Solanaceae* gehörigen Pflanze, die für *Phytophthora* anfällig ist, eine reduzierte Expression dieser Gene haben, wobei

- die Pflanze eine Kartoffel ist, die *Phytophthora-Resistenz* eine Resitenz gegen *Phytophthora infestans* ist und die Kombination von wenigstens zwei Genen Gene sind, die Proteine gemäß der SEQ ID Nr. 1 und der SEQ ID Nr. 2 codieren oder Proteine mit einer Sequenzidentität mit der SEQ ID Nr. 1 und der SEQ ID Nr. 2 von wenigstens 90%; oder
- die Pflanze eine Petunie ist, die *Phytophthora-Resistenz* eine Resistenz gegen *Phytophthora nicotianae* ist und die Kombination von wenigstens zwei Genen Gene sind, die Proteine gemäß der SEQ ID Nr. 3 und der SEQ ID Nr. 4 codieren oder Proteine mit einer Sequenzidentität mit der SEQ ID Nr. 3 und der SEQ ID Nr. 4 von wenigstens 90%; oder
- die Pflanze eine Tomate ist, die *Phytophthora-Resistenz* eine Resistenz gegen *Phytophthora infestans* ist und die Kombination von wenigstens zwei Genen Gene sind, die Proteine gemäß der SEQ ID Nr. 5 und der SEQ ID Nr. 6 codieren oder Proteine mit einer Sequenzidentität mit der SEQ ID Nr. 5 und der SEQ ID Nr. 6 von wenigstens 90%.

Revendications

1. Plante appartenant à la famille *Solanaceae* dans laquelle ladite plante comprend un trait génétique fournissant une résistance à *Phytophthora* et dans laquelle ledit trait de résistance est codé par une combinaison d'au moins deux gènes ayant une expression réduite desdits gènes en comparaison à ladite plante appartenant à la famille *Solanaceae* étant sensible à *Phytophthora,* dans laquelle :

   - ladite plante est la pomme de terre, ladite résistance à *Phytophthora* est une résistance à *Phytophthora infestans* et ladite combinaison des au moins deux gènes comporte des gènes codant pour des protéines selon SEQ ID N° 1 et SEQ ID N° 2 ou des protéines ayant une identité de séquence d'au moins 90 % avec SEQ ID N° 1 et SEQ ID N° 2; ou
   - ladite plante est le pétunia, ladite résistance à *Phytophthora* est une résistance à *Phytophthora nicotianae* et ladite combinaison d'au moins deux gènes comporte des gènes codant pour des protéines selon SEQ ID N° 3 et SEQ ID N° 4 ou des protéines ayant une identité de séquence d'au moins 90 % avec SEQ ID N° 3 et SEQ ID N° 4; ou
   - ladite plante est la tomate, ladite résistance à *Phytophthora* est une résistance à *Phytophthora infestans* et ladite combinaison d'au moins deux gènes comporte des gènes codant pour des protéines selon SEQ ID N° 5 et SEQ ID N° 6 ou des protéines ayant une identité de séquence d'au moins 90 % avec SEQ ID N° 5 et SEQ ID N° 6.

FIGURE 1

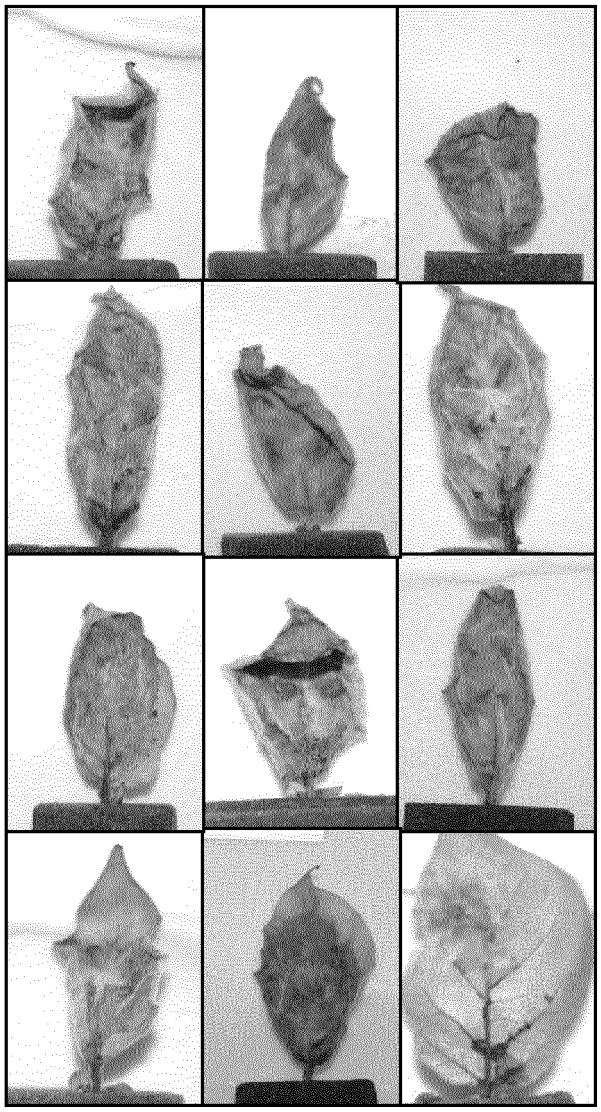


FIGURE 2a

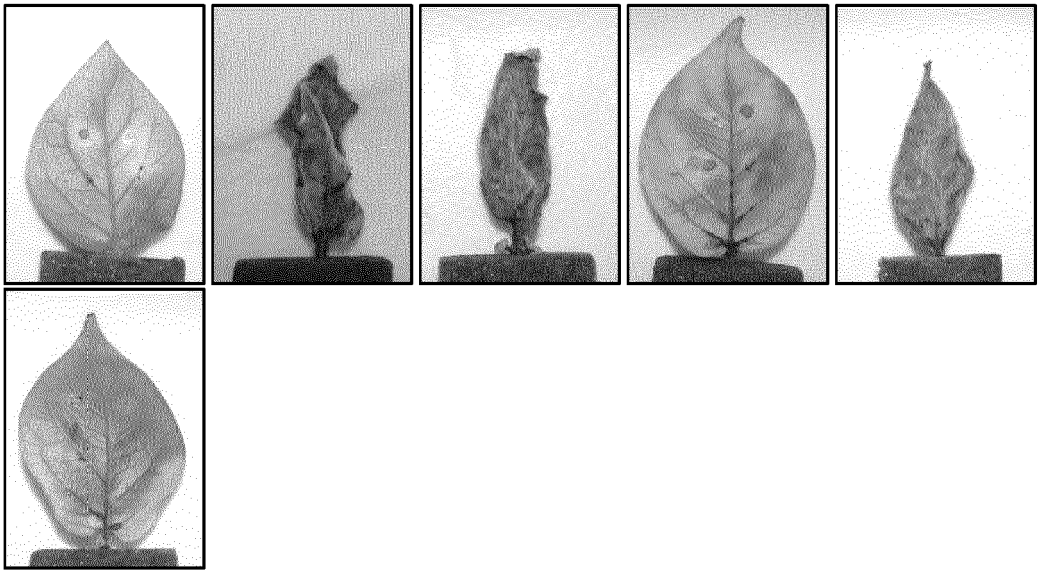


FIGURE 2b

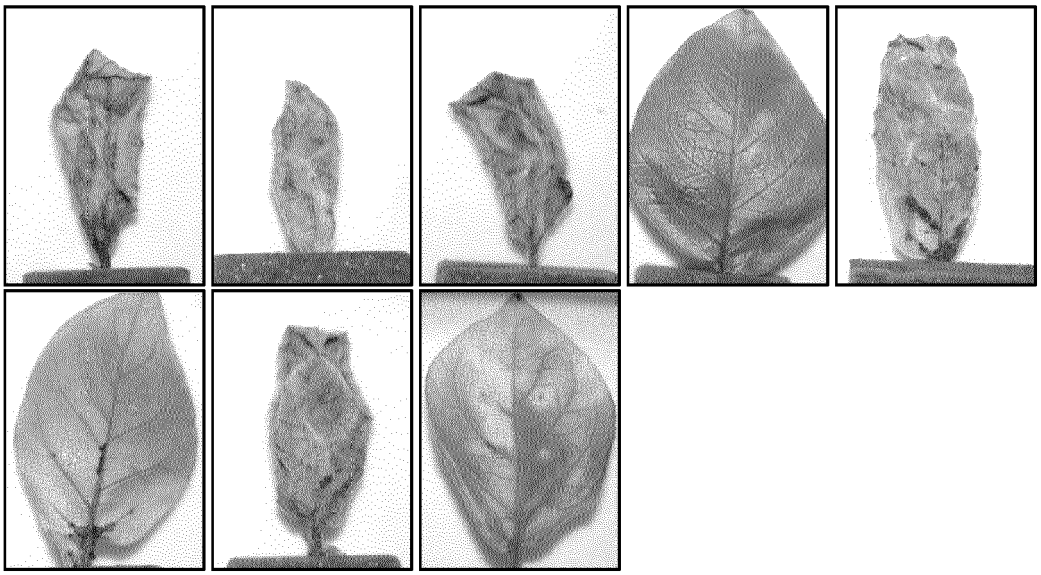


FIGURE 3

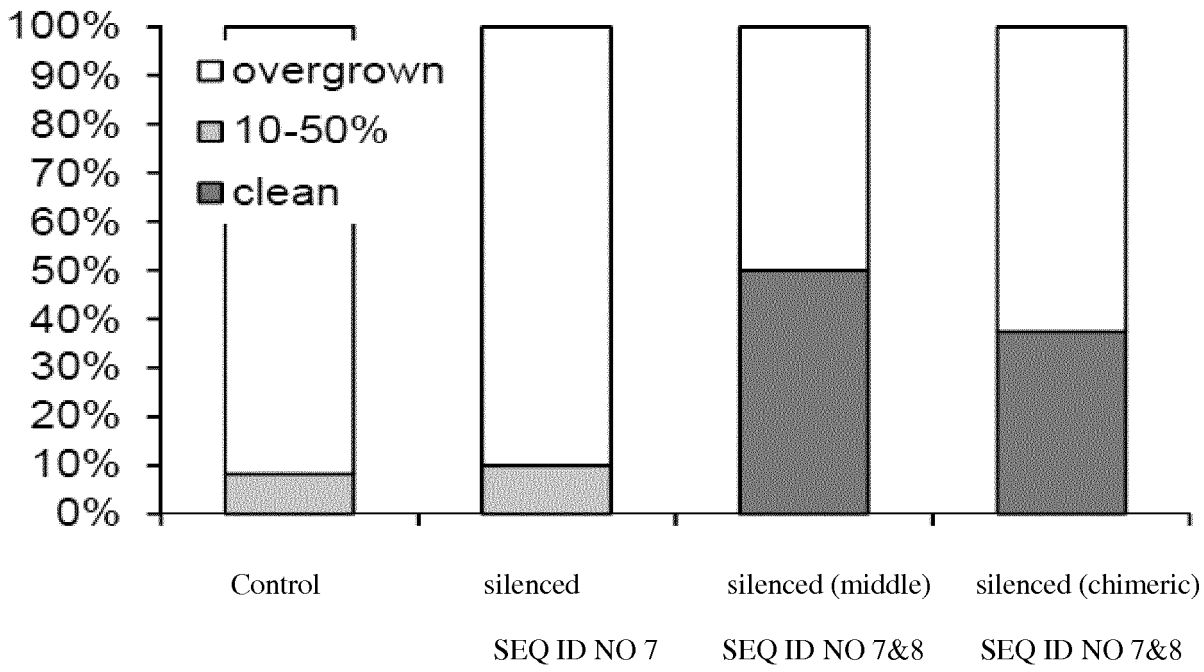

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
overgrown
10-50%
clean
Control
silenced
SEQ ID NO 7
silenced (middle)
SEQ ID NO 7&8
silenced (chimeric)
SEQ ID NO 7&8

FIGURE 4

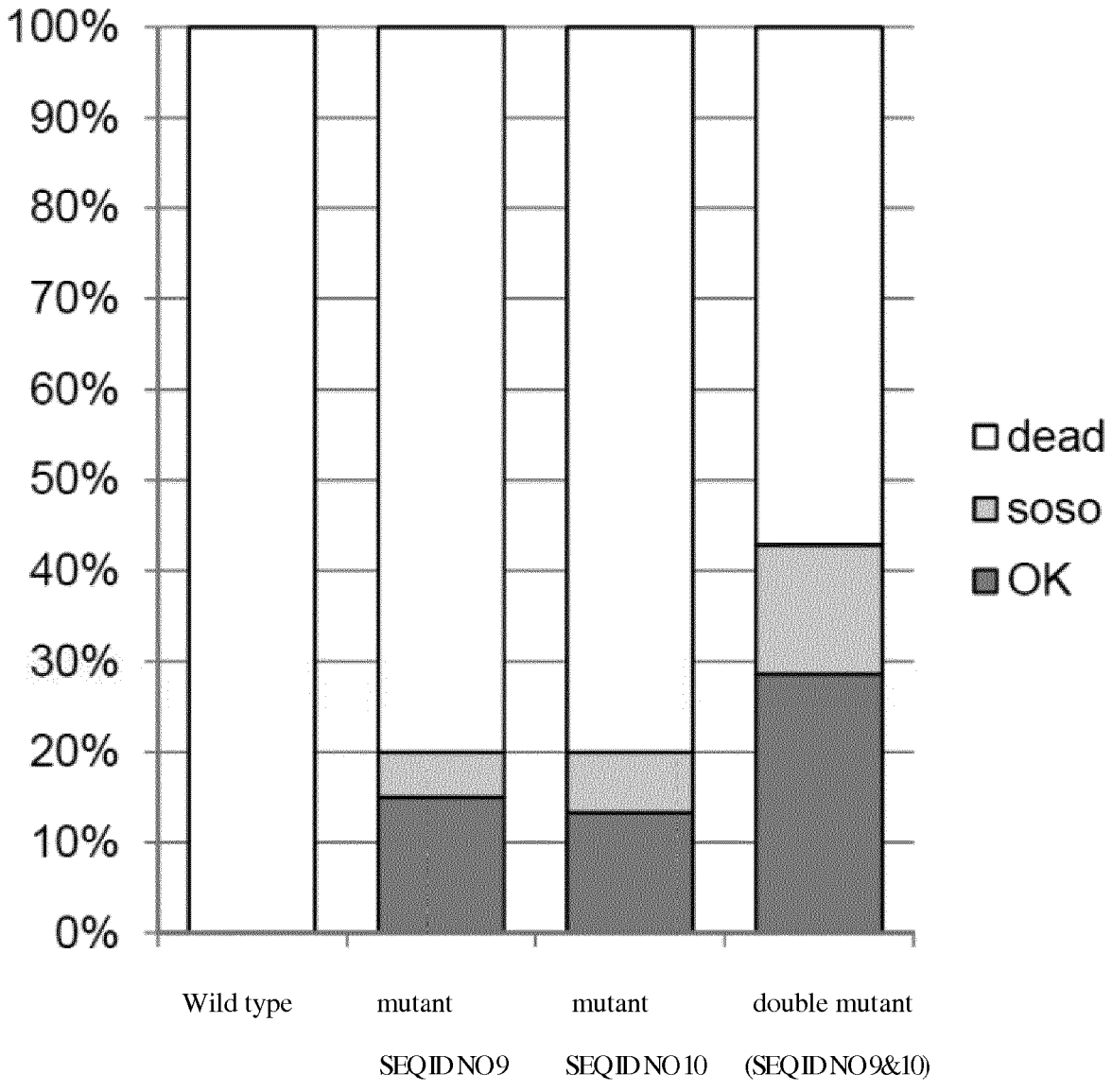
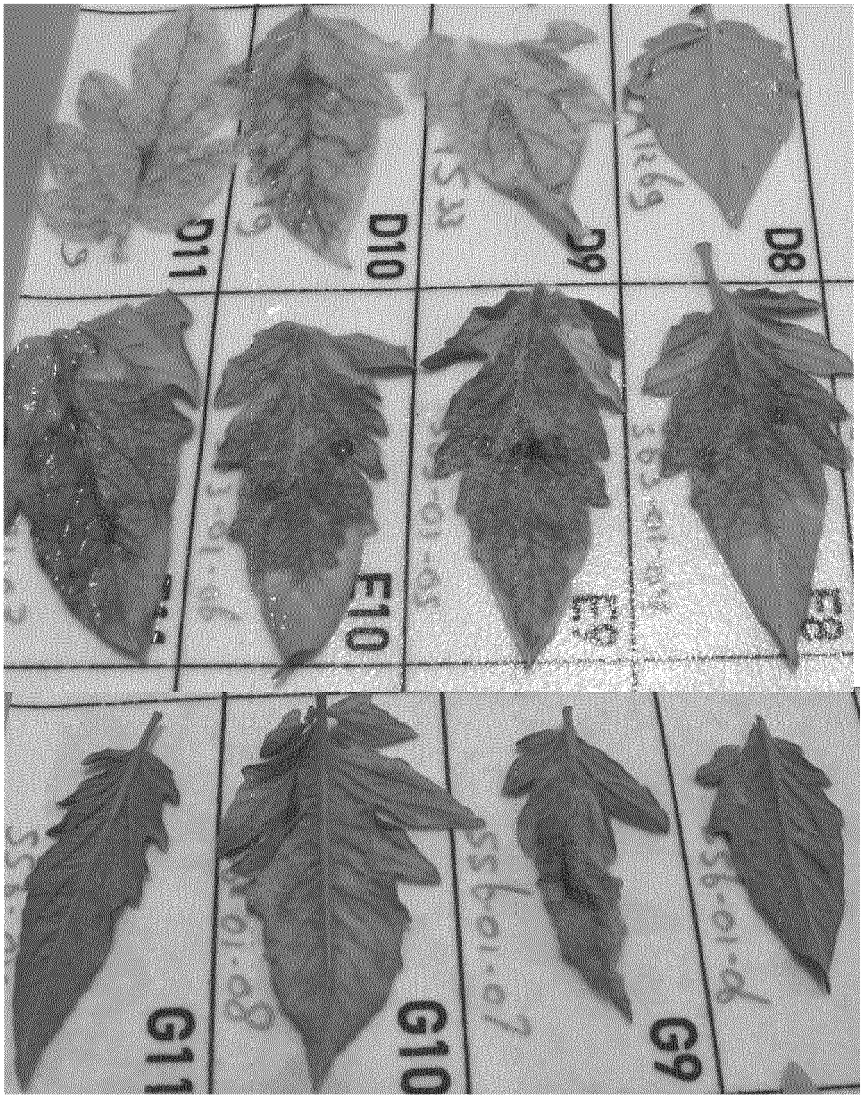

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
dead
soso
OK
Wild type
mutant
SEQ ID NO 9
mutant
SEQ ID NO 10
double mutant
(SEQ ID NO 9&10)

FIGURE 5



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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