



Plants resistant to pathogens and methods for production thereof

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PLANTS RESISTANT TO PATHOGENS AND METHODS FOR PRODUCTION THEREOF

FIELD OF THE INVENTION

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The invention relates generally to the field of agricultural biotechnology and plant diseases. In particular, the invention relates to plant genes involved in negative regulation of resistance to plant pathogens and uses thereof. More specifically, the invention relates to plants having a defective phytoalexin (PSK) function and exhibiting an increased resistance to plant pathogens. The invention also relates to methods for producing modified plants resistant to various diseases. Furthermore, the invention relates to plants having a defective PSK receptor (PSKR) function, and to methods of screening and identifying molecules that modulate PSKR expression or activity.

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BACKGROUND OF THE INVENTION

Plant pathogens represent a permanent threat on crop plants cultivation. In particular, infection of crop plants with bacteria, fungi, oomycetes or nematodes, can have a devastating impact on agriculture due to loss of yield and contamination of plants with toxins.

Most plant pathogenic bacteria belong to the following genera: *Ralstonia*, *Erwinia*, *Pectobacterium*, *Pantoea*, *Agrobacterium*, *Pseudomonas*, *Burkholderia*, *Acidovorax*, *Xanthomonas*, *Clavibacter*, *Streptomyces*, *Xylella*, *Spiroplasma*, and *Phytoplasma*. Plant pathogenic bacteria cause many different kinds of symptoms that include galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. Some plant pathogenic bacteria produce toxins or inject special proteins that lead to host cell death or produce enzymes that break down key structural components of plant cells. An example is the production of enzymes by soft-rotting bacteria that degrade the pectin layer that holds plant cells together. Still others, such as *Ralstonia* spp., colonize the water-conducting xylem vessels causing the plants to wilt and die. *Agrobacterium*

species even have the ability to genetically modify or transform their hosts and bring about the formation of cancer-like overgrowths called crown gall. Bacterial diseases in plants are difficult to control. Emphasis is on preventing the spread of the bacteria rather than on curing the plant. Cultural practices can either eliminate or reduce sources of bacterial contamination, such as crop rotation to reduce over-wintering. However, the most important control procedure is ensured by genetic host resistance providing resistant varieties, cultivars, or hybrids.

Nematodes are microscopic, worm-like organisms. They most commonly feed on plant roots, but some nematodes invade leaf tissue. Nematodes suck out liquid nutrients and inject damaging materials into plants. They injure plant cells or change normal plant growth processes. Symptoms of nematodes include swelling of stems or roots, irregular branching, deformed leaves, lack of blossoming and galls on roots. Nematodes can facilitate the entry of viruses and fungi into plants. Root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Globodera* spp. and *Heterodera* spp.) are the most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Currently, nematicides are the most important means of controlling nematodes. However, most of nematicides are non-specific, notoriously toxic and pose a threat to the soil ecosystem, ground water and human health. In the context of banning of most of these compounds, novel control measures are needed.

Oomycetes are fungus-like plant pathogens that are devastating for agriculture and natural ecosystems. *Phytophthora* species cause diseases such as dieback, late blight in potatoes, sudden oak death, and are responsible for severe crop losses (such as 30 % of the worldwide potato production). *Pythium* species are necrotrophs that kill plants and are responsible for pythiosis of crops, such as corn. Downy mildews, like *Plasmopara viticola* infecting grape, are biotrophic pathogens, which keep their hosts alive but weaken them in a way that severely affects yields. Downy mildews are easily identifiable by the appearance of white, brownish or olive "mildew" on the lower leaf surfaces. Oomycetes from the genus *Albugo* provoke white rust or white blister diseases on a variety of flowering plants. Oomycetes were long time considered as fungi, because they are heterotrophic, mycelium-forming organisms. However, several morphological

and biochemical characteristics discriminate oomycetes from fungi. Current taxonomy clusters oomycetes with photosynthetic organisms like brown algae or diatoms within the kingdom of stramenopiles. Due to their particular physiological characteristics, no efficient treatments against diseases caused by these microorganisms are presently available. Pesticides currently used against oomycetes rely on the phenylamide metalaxyl, which inhibits RNA polymerase-1. Metalaxyl impacts the environment, and resistance of the pathogens to this oomycide develop rapidly, now being a general characteristic of pathogenic *P. infestans* and *P. capsici* populations from potato and pepper, respectively.

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Common fungal diseases include powdery mildew, rust, leaf spot, blight, root and crown rots, damping-off, smut, anthracnose, and vascular wilts. Currently, fungal diseases are controlled for example by applying expensive and toxic fungicidal, chemical treatments using, e.g., probenazole, tricyclazole, pyroquilon and phthalide, or by burning infected crops. These methods are only partially successful since the fungal pathogens are able to develop resistance to chemical treatments.

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To reduce the amount of pesticides used, plant breeders and geneticists have been trying to identify disease resistance loci and exploit the plant's natural defense mechanism against pathogen attack.

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Plants can recognize certain pathogens and activate defense in the form of the resistance response that may result in limitation or stopping of pathogen growth. Many resistance (R) genes, which confer resistance to various plant species against a wide range of pathogens, have been identified. However, the key factors that switch these genes on and off during plant defense mechanisms remain poorly understood. Furthermore, pathogens may mutate and overcome the protection conferred by resistance genes. To control late blight disease, introgression of dominant resistance genes into susceptible cultivars has frequently been used to manage *Phytophthora* resistance. Eleven R genes from the wild potato species, *Solanum demissum* have been introduced into modern potato cultivars. However, *P. infestans* races quickly evaded the new single gene-mediated resistance properties of the cultivars. R gene introgression thus has shown its

limits for *Phytophthora* resistance breeding, and alternative programs have to be developed to render oomycete resistance durable.

Phytosulfokine (PSK) is a secreted peptide that has been first identified in the medium
 5 derived from asparagus (*Asparagus officinalis* L.) mesophyll culture and was proposed to be the main chemical factor responsible for “conditioning” or “nursing” i.e., the growth-promoting effects triggered by culture media previously used for cell culture or by physically separated “feeder” cells (Matsubayashi and Sakagami, 1996).

10 PSK peptides were also isolated from conditioned medium derived from rice (*Oryza sativa* L.) suspension cultures and identified to be present in two forms: a sulfated pentapeptide ([H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-Gln-OH], PSK α) and its C-terminal-truncated tetrapeptide ([H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-OH], PSK β) (Matsubayashi Y. *et al.*, 1997). The authors have suggested that a signal transduction pathway mediated
 15 by PSK peptide factors is involved in plant cell proliferation. PSK is produced from about 80 amino acids long precursor peptides via post-translational sulfation of tyrosine residues and proteolytic processing (Yang *et al.*, 1999). Genes encoding PSK precursors are redundantly distributed in the genome and are expressed in cultured cells and in a variety of tissues, including leaves, stems, flowers and roots (Matsubayashi Y. *et al.*,
 20 2006; Kutschmar *et al.*, 2008).

Two PSKR receptors have been identified in different plant species: PSKR1 and PSKR2. These receptors are members of the leucine-rich repeat receptor kinase (LRR-RK) family. PSK interacts with its receptor in a highly specific manner with a
 25 nanomolar dissociation constant. Furthermore, the PSK binding domain of carrot PSKR1 (DcPSKR1) has been identified by photoaffinity labeling (Shinohara *et al.*, 2007). The authors have found that deletion of Glu503-Lys517 completely abolishes the ligand binding activity of DcPSKR1. This region is in the island domain flanked by extracellular LRRs, indicating that this domain forms a ligand binding pocket that
 30 directly interacts with PSK.

PSK is mainly known as an endogenously secreted, sulfated 5-amino-acid peptide that is a key factor regulating cellular dedifferentiation and redifferentiation and that affects cellular potential for growth via binding to PSK receptor (PSKR). Recently, besides the mitogenic activity, an antifungal activity of PSK peptide has been suggested by Bahyrycz *et al.* (2008). This document shows that the PSK α and - β peptides inhibit *in vitro* the mycelium growth of *Phoma nareissi* and *Botrytis tulipae* pathogens in a dose-dependent manner.

Loivamaeki *et al.*, 2010 also propose a role of PSK signaling in wound formation in plants. Transcriptional activation of PSK/PSKR1 in crown galls is likely due to the cellular redifferentiation processes occurring during tumorigenesis. Activation of PSK signaling as a wound response has also been suggested by Motose *et al.*, Plant Physiol. 150, 437-447, 2009.

Amano *et al.*, 2007 concerns the identification of a new sulphated glycopeptide PSY1, related to phytosulphokines, and its involvement in developmental processes.

WO 02/083901 concerns a method of modifying growth, architecture, or morphology of a plant, based on the modulation of expression or activity of a GREP (Growth Regulating Protein) polypeptide or of a PSK homolog identified in rice, OsPSK.

PSK is thus essentially presented in the art as a regulator of cell proliferation or differentiation, with possible antifungal activity. There is no disclosure or suggestion in the art that PSK is a key regulator of pathogen resistance in plants.

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SUMMARY OF THE INVENTION

The present invention provides novel and efficient methods for producing plants resistant to pathogens. Surprisingly, the inventors have discovered that mutant plants with defective PSK and/or PSK receptor (PSKR) gene(s) are resistant to plant diseases while plants over-expressing the PSK or PSKR gene are more susceptible to plant diseases. The inventors have also demonstrated that such plants with a defective PSK or

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PSKR gene function acquire improved resistance to different types of pathogens, such as oomycete, nematode and bacterial pathogens, showing the broad application of this discovery.

- 5 An object of this invention therefore relates to plants comprising a defective PSK function. As will be discussed, said plants exhibit an increased or improved resistance to plant pathogens. Preferably, said plants are dicots, preferably selected from the families *Solanaceae* (e.g. tomato), *Liliaceae* (e.g. asparagus), *Apiaceae* (e.g. carrot), *Chenopodiaceae* (e.g. beet), *Vitaceae* (e.g. grape), *Fabaceae* (e.g. soybean),
 10 Cucurbitaceae (e.g. Cucumber) or *Brassicaceae* (e.g. rapeseed, *Arabidopsis thaliana*), or monocots, preferably selected from the cereal family *Poaceae* (e.g. wheat, rice, barley, oat, rye, sorghum or maize).

The invention more particularly relates to plants having a defective PSK peptide(s)
 15 and/or PSK receptor, preferably PSKR1 receptor, and exhibiting an increased resistance to plant pathogens.

Another particular object of this invention relates to plants comprising defective PSK genes and exhibiting an increased resistance to plant pathogens.

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A further particular object of this invention relates to plants comprising a defective PSKR gene and exhibiting an increased resistance to plant pathogens.

A further object of this invention relates to seeds of plants of the invention, or to plants,
 25 or descendents of plants grown or otherwise derived from said seeds.

A further object of the invention relates to a method for producing plants having increased resistance to plant pathogens, wherein the method comprises the following steps:

- 30 (a) inactivation of PSK and/or PSKR gene(s) in plant cells;
 (b) optionally, selection of plant cells of step (a) with defective PSK and/or PSKR gene(s);

- (c) regeneration of plants from cells of step (a) or (b); and
- (d) optionally, selection of a plant of (c) with increased resistance to pathogens, said plant having defective PSK or PSKR gene(s).

5 As will be further disclosed in the present application, the PSK function may be rendered defective by various techniques such as for example deletion, insertion and/or substitution of one or more nucleotides, site-specific mutagenesis, ethyl methanesulfonate (EMS) mutagenesis, targeting induced local lesions in genomes (TILLING), EcoTILLING, knock-out techniques, or by gene silencing induced by RNA
10 interference. The PSK function may also be rendered defective by altering the activity of the PSK peptide or receptor, e.g., using specific antibodies or a soluble receptor.

The invention also relates to a method for conferring or increasing resistance to plant pathogens to a plant, comprising a step of inhibiting permanently or transiently the PSK
15 function in said plant or an ancestor thereof, e.g., by inhibiting the expression of the PSK gene(s) and/or the PSKR gene(s) in said plant.

The invention also relates to a method for protecting plants against pathogens, comprising a step of inhibiting permanently or transiently the PSK function in said plant
20 or an ancestor thereof, e.g., by inhibiting the expression of the PSK gene(s) and/or the PSKR gene(s) in said plant.

The invention also relates to a method for decreasing pathogen proliferation in a plant, comprising a step of inhibiting permanently or transiently the PSK function in said plant
25 or an ancestor thereof, e.g., by inhibiting the expression of the PSK gene(s) and/or the PSKR gene(s) in said plant.

Another object of this invention relates to an inhibitory nucleic acid, such as an RNAi, an antisense nucleic acid, or a ribozyme, that inhibits the expression (e.g., transcription
30 or translation) of the PSK and/or PSKR gene(s). Another object of the invention relates to the use of such nucleic acid for increasing resistance of plants or plant cells to plant

pathogens and/or for decreasing plant pathogen proliferation in plants or plant cells and/or for protecting plants or plant cells against plant pathogens.

The invention also relates to methods of identifying molecules that modulate the PSKR gene expression, the method comprising:

- (a) providing a cell comprising a nucleic acid construct that comprises a PSKR gene promoter sequence operably linked to a reporter gene;
- (b) contacting the cell with a candidate molecule;
- (c) measuring the activity of PSKR promoter by monitoring of the expression of a marker protein encoded by the reporter gene in the cell;
- (d) selecting a molecule that modulates the expression of the marker protein.

Preferably, the selected molecules inhibit the expression or the activity of PSKR, preferably PSKR1.

The invention also relates to uses of the molecules selected according to the above methods for increasing resistance of plants to plant pathogens and/or for decreasing plant pathogen proliferation in plants or plant cells and/or for protecting plants or plant cells against plant pathogens.

The invention also relates to an antibody that specifically binds a PSK peptide or receptor, or a fragment or derivative of such antibody having essentially the same antigenic specificity, as well as to the use thereof to improve or cause pathogen resistance in plants and/or for decreasing plant pathogen proliferation in plants or plant cells and/or for protecting plants or plant cells against plant pathogens.

The invention is applicable to produce legumes, vegetables and cereals having increased resistance to pathogens, and is particularly suited to produce resistant tomato, potato, asparagus, carrot, beet, rapeseed, grape, wheat, rice, barley, oat, rye, sorghum or maize.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Constitutive expression of the *PSK2* gene. The expression of the *PSK2* gene (transgenic *Arabidopsis* line *PSK2pro:GFP:GUS*) is developmentally regulated. **(A-E):** *PSK2* expression in the root system. **(A,B)** GUS activity **(A)** and GFP **(B)** revealing *PSK2* promoter activation is detectable in the root tips (lateral root cap) but not in the elongation zone. **(C,D)** In fully differentiated roots, *PSK2* expression localizes to the vascular cylinder. **(E)** Expression of *PSK2* in lateral root primordial; **(F-I):** *PSK2* expression in the shoots is localized in the vascular system of leaves and cotyledons **(F)**, trichomes **(G)**, and stomata **(H,I)**. All analyses were performed on 2 week-old seedlings.

Figure 2: *PSK* gene expression patterns in *Arabidopsis thaliana* after nematode and oomycete infection. **(2A)** Expression profiling of *PSK* genes was analyzed by microarray hybridizations. Samples were prepared from isolated galls and infected cotyledons at different time points after infection with *M. incognita* and *H. arabidopsidis*, respectively. Represented are mean Log2 ratios between infected- and uninfected tissues for two biological replicates. nc, not changed. **(2B)** Relative *PSK* transcript accumulations in *Arabidopsis* galls at 7 (white bars), 14 (grey bars), and 21 (black bars) days after nematode inoculation (DAI) by quantitative RT-PCR in comparison to uninfected roots. Shown is a representative experiment giving mean values ($\pm$ SD) from 3 technical replicates. **(2C)** *PSK2* expression pattern in galls of *M. incognita*-infected roots of the transgenic *Arabidopsis* line *PSK2pro:GFP:GUS*. **A,B.** Reduced GUS activity is revealed in the center of developing galls. **C.** GFP signal was not detected in nematode feeding cells in projections of serial confocal optical *in vivo* sections. *, giant cell; n, nematode.

Figure 3: Developmentally regulated expression of the *PSKR1* gene. **(A)** In the transgenic *Arabidopsis* line *PSKR1pro:GFP:GUS*, GUS activity revealing *PSKR1* promoter activation is detectable in differentiated root tissues and the root cap, but not in the dividing and elongation zone. **(B)** Constitutive *PSKR1* transcription in root cells, as monitored through GFP fluorescence. **(C)** Transcription of *PSKR1* occurs in the root and the transition zone, but not in the hypocotyl. **(D-E)** GFP fluorescence in the

epidermis of cotyledons localizes to stomata. All analyses were performed with 2 week-old seedlings.

Figure 4: *PSKR1* gene expression pattern in *Arabidopsis thaliana* after oomycete infection. (4A) *PSKR1* transcript abundance was analyzed by qRT-PCR at different time points after spray-treatment of *Arabidopsis* (ecotype Ws-0) cotyledons with water, or with conidiospore suspensions at 40,000 spores/ml of the downy mildew pathogen, *Hyaloperonospora arabidopsidis* (Hpa). Shown are means ($\pm$ SD) from 3 technical replicates normalized for values from 2 reference genes (At5g62050 and At5g10790), as calculated by the qBase1.3.5 software. The experiments performed with samples from two biological replicates gave similar tendencies. Dpi: Days post inoculation. **(4B)** Transcriptional activation of *PSKR1* in response to Hpa infection, as monitored through the GUS reporter gene activity in the transgenic *Arabidopsis* line *PSKR1*pro:GFP:GUS. Before inoculation, constitutive expression of *PSKR1* is visible through GUS activity in cotyledons at time point 0. Upon inoculation, expression increases continuously and localizes to infected areas of the mesophyll.

Figure 5: *PSKR1* gene expression pattern in *Arabidopsis thaliana* after nematode infection. (5A) *PSKR1* transcript analysis by qRT-PCR at 7 (white bars), 14 (gray bars), and 21 (black bars) days after inoculation (DAI). Two biological replicates were performed. The bars represent mean values ($\pm$ SD) from two independent experiments. **(5B)** Expression pattern of the GFP reporter gene under control of the *PSKR1* promoter in galls of the transgenic *Arabidopsis* line *PSKR1*pro:GFP:GUS, which were induced by *M. incognita* in roots, 7 (A) and 21 (B) DAI with 150 surface-sterilized freshly hatched *M. incognita* J2 larvae.

Figure 6: A *psk3* knock-out mutant is less susceptible to oomycete infection. (6A) Schematic illustration of the genomic organization of *PSK3* (locus At3g44735), primer attachment sites, and T-DNA insertion and orientation in genomic DNA from line *psk3-1* (SAIL_378_F03). Bars represent exons and lines correspond to introns (between exons) and untranslated sequences (at the 5'end and at the 3'end). The T-DNA insertion localizes within the third exon. Amplicons revealing the *PSK3* transcript are not detected in the mutant line, thus confirming the molecular knock-out phenotype. Amplification of the constitutively expressed *EF1 α* gene (At1g07930) transcript

showed that similar amounts of intact cDNAs were used for RT-PCR experiments. **(6B)** Quantitative analysis for the interaction phenotype of the *PSK3* knockout mutant with *H. arabidopsidis*. Sporulation of *H. arabidopsidis* isolate Noco2 on cotyledons of the *Arabidopsis psk3-1* mutant is reduced by >50 %, when compared to wild-type plants
 5 (Col-0). Plantlets were collected 7 days post inoculation in 1 ml of water, vortexed, and the titer of liberated conidiospores was determined with a hemocytometer. For statistics, 20 samples at 10 plantlets were prepared for each line and analysis. The bars represent mean values ($\pm$ SD). The experiment was repeated 3 times with similar results. Statistically significant differences for values compared with the wild type were
 10 determined by Student's t-test (*** $P < 0.0001$).

Figure 7: Over-expression of the *PSK2* or *PSK4* gene increases susceptibility to *H. arabidopsidis*, *M. incognita*, and *R. solanacearum*. **(7A)** Quantitative analysis for the interaction phenotype with *H. arabidopsidis* of transgenic lines overproducing *PSK2* (*Arabidopsis* line p35S:*PSK2*) and *PSK4* (*Arabidopsis* line p35S:*PSK2*). The bars
 15 represent mean values ($\pm$ SD). The experiment was repeated 3 times with similar results. Statistically significant differences for values compared with the wild type were determined by Student's t-test (*** $P < 0.0001$). **(7B)** Root knot nematode infection is significantly stimulated in the transgenic lines constitutively overexpressing *PSKs*. *Arabidopsis* plants were infected *in vitro* 14 d after germination with 150 surface-
 20 sterilized freshly hatched *M. incognita* J2. Statistically significant differences were determined by the Student's t test (* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$). **(7C)** Bacterial multiplication is strongly enhanced in transgenic lines constitutively overexpressing *PSKs*. Four week-old plants were root-inoculated with a solution containing 10^7 bacteria per ml of the virulent bacterial isolate RD15. For analyzing bacterial internal growth,
 25 the aerial parts of three inoculated plants were weighed and ground in a mortar after addition of sterile water (2.0 ml per g of fresh weight). Various dilutions of the ground material were then performed with sterile water and 3 x 40 μ l of bacterial suspensions were spotted on petri plates containing solid SMSA medium (Elphinstone *et al.*, 1996), and grown at 30°C. For each time point, triplicate assays were performed for each *A.*
 30 *thaliana* line. The bars represent mean values ($\pm$ SD).

Figure 8: The *pskr1* knock-out mutants are less susceptible to infection by *H. arabidopsidis*. (8A) Schematic illustration of the genomic organization of *AtPSKR1* (locus At2g02220), primer attachment sites, and T-DNA insertions and orientations in genomic DNA. (8B) RT-PCR revealed *PSKR1* transcripts in wild-type *Arabidopsis* (Col-N8846, Ws, Col-0, and Col-8 CS60000). Amplification of transcripts from the constitutively expressed *AtEF1 α* gene (At1g07930) show that similar amounts of intact cDNAs were used for RT-PCR experiments. (8C) Allelic *pskr1* mutants show reduced *H. arabidopsidis* sporulation. For statistics, 20 samples at 10 plantlets were prepared for each line and analysis. The bars represent mean values ($\pm$ SD), and *** indicates significant differences between wild-type and mutant lines with $P < 0.0001$, as determined by Student's t-test. All experiments were repeated 3 times and gave similar results. 1-1, 1-2, 1-3, and 1-4 represent the mutants *pskr1-1*, *pskr1-2*, *pskr1-3*, and *pskr1-4*, respectively.

Figure 9: The *pskr1* knock-out mutants are less susceptible to infection by *M. incognita*. The nematode infects roots and initiates gall formation to a similar extent in *pskr1* mutants and wild-type plants, as analyzed 10 days post inoculation (Dpi). A reduction in the amount of mature galls is observed in *pskr1* mutants at 21 Dpi. The inhibition of nematode development in the absence of PSKR1 becomes most evident during the parthenogenetic production of egg masses, which are strongly reduced on *pskr1* mutants at 75 Dpi. Data represent means ($\pm$ SD) from at least two experiments in which a minimum of 50 seedlings of each line were evaluated for nematode infection. *** represents statistically significant differences with $P < 0.0001$, as determined by Student's t-test.

Figure 10: The *pskr1* knock-out mutants are less susceptible to infection by *R. solanacearum*. Plants with a Ws (A) and Col (B) genetic background were root-inoculated with the virulent bacterial isolates RD15 and GMI1000, respectively. Approximately 2 cm were cut from the bottom of the Jiffy pots and the exposed roots of the plants were immersed for 3 min in a suspension containing 10^7 bacteria per ml. The plants were then transferred to a growth chamber with a day/night cycle of 8 h at 27°C, 120-140 μ E m⁻²s⁻² and 16 h at 26°C, respectively, keeping relative humidity at 75 %. Disease symptoms on inoculated plants were scored at 3, 4, 5, 6, and 7 days post

inoculation according to a disease index (DI) covering DI 0 (no wilt), and DI 1, DI 2, DI 3, and DI 4, representing 25 %, 50 %, 75 %, and 100% of wilted leaves, respectively. Shown is a representative experiment among several repetitions with similar results, giving means ($\pm$ SD) from inoculations of at least 28 plants/line. All *pskr1* mutants are significantly less susceptible during the exponential bacterial growth phase between 3 and 5 days post inoculation with $P < 0.0001$. The Col genetic background (B) of *A. thaliana* shows an overall higher susceptibility to *R. solanacearum*, and the effect of the *pskr1* mutation is most pronounced in *pskr1-2* in the Ws genetic background (A). Full susceptibility to *R. solanacearum* was restored through the introduction of a fully functional *PSKR1* gene into the *pskr1-2* genetic background (complemented *Arabidopsis* line *Cppskr1-2*, compare legend to Figure 11). An acceleration of disease at late time points of infection was observed in the line overexpressing *PSKR1* under the control of the constitutive 35S promoter (overexpressing line *PSKR1-OE*, compare legend to Figure 11).

Figure 11: Reduced susceptibility of *pskr* mutants is reverted by expression of a functional *PSKR* gene. Overexpression of the *PSKR* gene increases susceptibility to *H. arabidopsidis*. Downy mildew susceptibility correlates with *PSKR1* expression (A) Conidiospores/mg FW levels obtained in the *pskr1-2* mutant and transgenic lines obtained after infection with *H. arabidopsidis*. The mutant phenotype of *pskr1-2* (Ws-0 background) is fully reverted in line *Cppskr1-2* through complementation with a genomic 5,472 bp fragment comprising the 1,771 bp region 5' of the translation initiation codon, 3027 bp of entire coding sequence and 650 bp of 3' non-translated region of At2g02220. The genomic fragment was amplified by PCR, cloned into the Gateway destination vector pHGW (Karimi *et al.*, 2002), and transferred into *pskr1-2* by *Agrobacterium*-mediated transformation. Overexpression of *PSKR1* in the Ws-0 wild-type (line *PSKR1-OE*) increases downy mildew susceptibility by almost 100 %. For overexpression of the gene, 3,060 bp of the coding region including Start and Stop codons were amplified from genomic DNA, cloned into the Gateway destination vector pH2GW7 (Karimi *et al.*, 2002), and mobilized into *Arabidopsis* by *Agrobacterium*-mediated transformation. The pathogen assays were performed as described before. The bars represent mean values ($\pm$ SD), and *** indicates significant differences between wild-type and mutant lines with $P < 0.0001$, as determined by Student's t-test. All

experiments were repeated 3 times and gave similar results. **(B)** Expression levels of *PSKR1* in the different mutant and transgenic lines obtained after infection with *H. arabidopsidis*. Relative *PSKR1* transcript accumulations in *Arabidopsis* seedlings (15 days after sowing) were determined by quantitative real time RT-PCR. Expression ratios were calculated using the $2^{-(\Delta\Delta CT)}$ method with *UBP22* (At5g10790) for normalization and wild-type *PSKR1* expression as the reference. The bars ($\pm$ SD) represent mean values of three technical replicates.

Figure 12: Reduced disease susceptibility of *pskr1* mutants is not a consequence of constitutively activated, or pathogen-triggered defense responses. The activation of salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene (JA/ethylene)-mediated defense signaling pathways in *Arabidopsis* is independent of *PSKR1*. Marker genes for SA-, JA, and JA/ethylene-mediated signaling pathways were *PR1a* (At2g14610) *PDF1.2* (At5g44420), and *PR4* (At3g04720), respectively. Expression of these defense-related genes was analyzed by quantitative real-time RT-PCR in wild type (Ws), mutant (*pskr1-2*), and transgenic *PSKR1* overexpressor (*PSKR1-OE*) plants upon spray treatment of cotyledons with water, or with conidiospore suspensions (40,000 spores/ml) of the *H. arabidopsidis* isolate Emwa1. Samples for RNA extraction and qRT-PCR were prepared at time point 0, and 24, 48, 72, and 120 hours after onset of treatment. Relative quantities of marker gene transcripts were normalized with *AtOXA1* (At5g62050) and *AtUBP22* (At5g10790) using the Q-Base software. Represented are means ($\pm$ SD) from 3 technical replicates. Two independent experiments gave similar results.

Figure 13: *PSKR1* suppression causes reduced proliferation of *R. solanacearum*, *H. arabidopsidis*, and *M. incognita*. **(A, B)** Bacterial multiplication is strongly reduced in the absence of *PSKR1* in the *pskr1-2* mutant. For each time point, triplicate assays were performed for each *A. thaliana* line. The bars represent mean values ($\pm$ SD). A and B are representations of the same experimental results with bacterial titers given as absolute and log values, respectively. Bacterial multiplication was drastically reduced ($\sim$ 1,000-fold) in the *pskr1-2* mutant, restored in the complemented line *Cppschr1-2*, and increased ($\sim$ 2-fold) in the overexpressing line *PSKR1-OE*. **(C)** Oomycete hyphal development in leaf tissues is reduced in the absence of *PSKR1* in the *pskr1-2* mutant. Plants were spray-inoculated with 40,000 spores/ml and cotyledons were collected 5 days post

inoculation. The development of hyphae within infected cotyledons was visualised by trypan blue staining. A fully developed, branched hyphal network was observed in the Ws wild-type plants. The network and hyphal branching was strongly reduced in the absence of PSKR1 (line *pskr1-2*), but became aberrant upon overexpression of PSKR1 (line *PSKR1-OE*). Shown are representative transmission light micrographs. **(D)** The reduced egg mass production by *M. incognita* is a consequence of reduced giant cell sizes in the absence of PSKR1. For morphological analyses, nematode-infected roots of *pskr1-2*, *PSKR1-OE* and wild-type plants (ecotype Ws) were fixed in 2% glutaraldehyde in 50mM Pipes buffer (pH 6.9) on 7, 14 and 21 days post inoculation and then dehydrated and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany) as described by the manufacturer. Embedded tissues were sectioned (3µm) and stained in 0.05% toluidine blue, mounted in Depex (Sigma) and microscopy was performed using bright field optics. Images were collected with a digital camera (Axiocam; Zeiss). Tissue sections through galls on 7 days post inoculation from *pskr1-2* and *PSKR1-OE* showed no difference in gall and giant cells formation in comparison with control. At later stages of gall development (14 and 21 days post inoculation) the giant cells from *pskr1-2* mutant plants were significantly smaller. For giant cell surface measurements, serial sections stained with toluidine blue were examined using the AxioVision V 4.8.1.0 software. The three biggest giant cells per gall from at least 50 galls per phenotype were chosen for measurements. Galls from *pskr1-2* mutant plants contain significantly smaller giant cells in comparison to control plants at 14 days post inoculation.

Figure 14: Representation of tomato mutations within *SIPSKR1* identified following TILLING strategy. The genomic regions of *SIPSKR1* which have been targeted in the TILLING method are indicated by arrows Target 1 and Target 2. The six mutations identified with the TILLING approach are the following: *pskr1.1* A88 T, *pskr1.2* T119 C, *pskr1.3* G502 A, *pskr1.4* G856 A, *pskr1.5* G2285 A and *pskr1.6* G1978 A. The drawing also represents protein domains as bottom arrows indicating the signal peptide (SP), the leucine-rich repeat domain (LRR), the transmembrane domain (TM), and the kinase domain. Primer attachment sites for TILLING are indicated in capital letters,

DETAILED DESCRIPTION OF THE INVENTION

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The present invention provides novel and efficient methods for producing plants resistant to pathogens, having defective PSK and/or PSK receptor functions.

Surprisingly, the inventors have now discovered that PSKs act as negative regulators of plant resistance to plant pathogens, i.e., their inhibition increases resistance by reducing susceptibility. To our knowledge, this is the first example of a negative regulation of resistance in plants by growth factors. The PSK signaling pathway thus represents a novel and highly valuable target for producing plants of interest with increased resistance to pathogens. The inventors have further demonstrated that plants having defective PSK and/or PSK receptor functions have reduced susceptibility to different types of pathogens, such as oomycete, nematode and bacterial pathogens, showing the broad application of this invention.

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The present disclosure will be best understood by reference to the following definitions:

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Definitions

As used therein, the term “PSK peptide” designates a sulfated phytosulfokine peptide acting as a negative regulator of plant resistance. Such a PSK peptide preferably comprises the amino acid sequence of H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-OH (SEQ ID NO: 1) or the amino acid sequence of H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-Gln-OH (SEQ ID NO: 2), or any natural variant thereof (e.g., variants present in other plants or which result from polymorphism). Preferably, a PSK peptide contains at least 4 amino acids. More preferably, a PSK peptide contains at least 5 amino acids. Typically, a PSK peptide contains at least two sulfated amino acid residues, which are preferably tyrosine residues. The term PSK peptide also designates any precursor or immature form of the peptide, such as for example PSK preproteins comprising amino acid sequences of SEQ ID NO: 3, 4, 5, 6 or 7. Specific examples of PSK precursors include *Populus*

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trichocarpa PSK precursors comprising a sequence selected from SEQ ID NO: 8-13, *Oryza sativa* PSK precursors comprising a sequence selected from SEQ ID NO: 14-19, 95, 97, 99, 101, 103, *Vitis vinifera* PSK precursors comprising a sequence selected from SEQ ID NO: 20-24 and *Solanum lycopersicum* precursors comprising a sequence
5 selected from SEQ ID NO: 69, 71, 73 or 75.

Within the context of the present invention, the term “PSK gene” designates any nucleic acid that codes for a PSK peptide (or its precursor). The term “PSK gene” includes PSK DNA (e.g., genomic DNA) and PSK RNA (e.g., mRNA), as applicable. In particular, a
10 “PSK gene” includes any nucleic acid encoding a phytosulfokine peptide or a natural variant of such a peptide, as defined above. Examples of PSK genes include the PSK genomic DNA or RNA of *Arabidopsis thaliana*, *Solanum lycopersicum* (*Lycopersicon esculentum*), *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Triticum aestivum*, *Asparagus officinalis*, *Brassica napus*, *Beta vulgaris*, *Solanum tuberosum*, *Glycine max*, *Vitis*
15 *vinifera* and *Daucus carota*. Specific example of a PSK gene comprises the nucleic acid sequence of SEQ ID NO: 25-29, 86-90 (*Arabidopsis thaliana*), SEQ ID NO: 68, 70, 72 or 74 (*Solanum lycopersicum*), SEQ ID NO: 94, 96, 98, 100, 102, 104, 105, (*Oryza sativa*).

20 Further examples of PSK genes or peptides are listed below:

Rice (*Oryza sativa*)

GenBank: BAF11381.2, Os03g0232400

NCBI Reference Sequence: NP_001050886.1, Swiss-Prot: Q9FRF9.1 Q9FRF9, PSK3

GenBank: AAG46077.1

25 GenBank: BAF12800.1

GenBank: EEC75912.1, hypothetical protein OsI_12987

GENE ID: 4333708 Os03g0675600

GenBank: ABF98161.1, Phytosulfokines 3 precursor, putative

GenBank: EAZ28113.1, hypothetical protein OsJ_12080

30 Maize (*Zea mays*)

GenBank: ACG49207.1, PSK4

GenBank: DAA00297.1, PSK

NCBI Reference Sequence: NP_001105796.1, PSK1

GenBank: ACG23972.1, PSK

GenBank: ACG41544.1, phytosulfokine precursor protein

GenBank: ACG27399.1, phytosulfokine precursor protein

5 Sorghum (Sorghum bicolor)

GENE ID: 8085257 SORBIDRAFT_01g042120

GENE ID: 8084300 SORBIDRAFT_02g001950

GenBank : EES08686.1 SORBIDRAFT_05g021760

Wheat (Triticum aestivum)

10 GenBank : DAA00296.1, putative phytosulfokine peptide precursor

GenBank : ABG66637.1, phytosulfokine-alpha 2 precursor

GenBank: ABG66638.1, phytosulfokine-alpha 2 precursor

Wild Asparagus (Asparagus officinalis)

Swiss-Prot: Q9FS10, PSK

15 GenBank: BAB20706.1, preprophytosulfokine

Rapeseed (Brassica napus)

GenBank: DAA00277.1, putative phytosulfokine peptide precursor

Beet (Beta vulgaris)

Swiss-Prot: CAK22422.1, phytosulfokine-alpha peptide precursor

20 Tomato (Solanum lycopersicum)

GenBank: DAA00287.1, PSK4

Potato (Solanum tuberosum)

GenBank: DAA00294.1, PSK

GenBank: DAA00293.1, PSK

25 Soybean (Glycine max)

GenBank: ACU23402.1, phytosulfokine peptide precursor

GenBank: DAA00280.1, putative phytosulfokine peptide precursor

GenBank: DAA00283.1, putative phytosulfokine peptide precursor

GenBank: DAA00282.1, putative phytosulfokine peptide precursor

30 GenBank: DAA00279.1, putative phytosulfokine peptide precursor

Grape (Vitis vinifera)

GenBank: CBI38497.3, PSK

- GenBank: CAN65538.1 and CBI25131.3, PSKs
 GenBank: CBI19372.1, PSK
 GenBank: CBI30250.3, unnamed protein product
 GenBank: CBI17083.3
- 5 GenBank: CAN62427.1, hypothetical protein
Banana (*Musa acuminata*)
 GenBank: ABF70025.1, phytosulfokine family protein
Zinnia (*Zinnia violacea*)
 Swiss-Prot: Q8H0B9, preprophytosulfokine
- 10 Tree cotton (*Gossypium arboreum*)
 GenBank: DAA00278.1, putative phytosulfokine peptide precursor
Poplar (*Populus trichocarpa*)
 NCBI Reference Sequence: XP_002320667.1, PSK
 GenBank: EEE98982.1, PSK
- 15 NCBI Reference Sequence: XP_002320021.1, PSK
 NCBI Reference Sequence: XP_002301142.1, PSK
 GenBank: EEE87877.1
Pine tree (*Pinus taeda*)
 GenBank: DAA00289.1, PSK
- 20 Douglas fir (*Pseudotsuga menziesii*)
 GenBank: ACH59688.1
 GenBank: ACH59689.1
 GenBank: ACH59690.1
 GenBank: ACH59691.1
- 25 GenBank: ACH59692.1
 GenBank: ACH59693.1
 GenBank: ACH59694.1
 GenBank: ACH59695.1
 GenBank: ACH59696.1
- 30 GenBank: ACH59697.1
 GenBank: ACH59698.1
 GenBank: ACH59699.1

GenBank: ACH59701.1

GenBank: ACH59702.1

GenBank: ACH59703.1

GenBank: ACH59704.1

5 GenBank: ACH59705.1

GenBank: ACH59706.1

GenBank: ACH59707.1

GenBank: ACH59708.1

GenBank: ACH59709.1

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As used therein, the term “PSKR” or “PSK receptor” designates a receptor of a PSK peptide. Typically, a PSKR has an extracellular domain binding the PSK peptide as defined above, and an intracellular signaling domain having a kinase activity. The PSKR has been isolated and cloned from various species, including *Arabidopsis*
 15 *thaliana*, *Solanum lycopersicum*, *Daucus carota*, *Oryza sativa*, and *Vitis vinifera*. Illustrative sequences of a PSKR are provided as SEQ ID NO: 30, 31 (*Arabidopsis thaliana*), SEQ ID NO: 32 (*Daucus carota*), SEQ ID NO: 33 (*Vitis vinifera*), SEQ ID NO: 111, 113 (*Populus trichocarpa*), SEQ ID NO: 34, 107, 109 (*Oryza sativa*) and SEQ ID NO: 35, 114 (*Solanum lycopersicum*). The preferred PSKR according to the
 20 invention is PSKR1 receptor.

A “PSKR gene” designates any nucleic acid that codes for a PSKR receptor. In particular, a “PSKR gene” may be any DNA or RNA encoding a receptor of the phytosulfokine peptide, as applicable. Specific examples of PSKR gene include a
 25 nucleic acid comprising the sequence of SEQ ID NO: 36 or 37, which encode the amino acid sequences of PSKR1 or PSKR2 of *Arabidopsis thaliana*. In another embodiment, “PSKR gene” codes for any natural variant or homolog of a PSKR1 or PSKR2 protein. Examples of PSKR gene include the PSKR gene or RNA of *Solanum lycopersicum*, *Daucus carota*, *Vitis vinifera*. Illustrative sequences are provided as SEQ ID NO: 38, 39,
 30 40, 67, 91, 92, 93, 108, 109, 110 or 112.

Within the context of the present invention, the term “pathogens” designates all pathogens of plants in general. More preferably the pathogens are fungal, oomycete, nematode or bacterial pathogens. In a particular embodiment, fungal pathogens are cereal fungal pathogens. Examples of such pathogens include, without limitation,
 5 *Magnaporthe*, *Puccinia*, *Aspergillus*, *Ustilago*, *Septoria*, *Erysiphe*, *Rhizoctonia* and *Fusarium* species.

In a more preferred embodiment, the pathogens are biotrophic or hemi-biotrophic oomycete pathogens selected from the genera of *Phytophthora*, *Peronospora*,
 10 *Hyaloperonospora*, and *Plasmopara*. The most preferred oomycete pathogens are *Hyaloperonospora arabidopsidis*, *Phytophthora parasitica*, *Phytophthora infestans*, *Phytophthora capsici* and *Plasmopara viticola*.

In another preferred embodiment, the pathogens are nematode pathogens. The most
 15 preferred nematode pathogens are *Meloidogyne* spp. (*M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. graminicola*), *Globodera* spp. and *Heterodera* spp.

In another preferred embodiment, the pathogens are bacterial pathogens. The most
 20 preferred bacterial pathogen is *Ralstonia solanacearum*.

Different embodiments of the present invention will now be further described in more details. Each embodiment so defined may be combined with any other embodiment or
 25 embodiments unless otherwise indicated. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

PSK- or PSKR-defective plants

As previously described, the present invention is based on the finding that *PSK* and
 30 *PSKR* genes are negative regulators of plant resistance to plant pathogens. The inventors have demonstrated that the inactivation of the *PSK* or *PSKR* gene(s) increases plant resistance to plant pathogens.

The present invention thus relates to methods for increasing pathogen resistance in plants based on a regulation of PSK pathways. The present invention also relates to methods of protecting a plant against pathogens by decreasing or suppressing PSK
5 function in said plant.

The invention also relates to plants or plant cells having a defective PSK function.

The invention also relates to constructs (e.g., nucleic acids, vectors, cells, etc) suitable
10 for production of such plants and cells, as well as to methods for producing plant resistant regulators.

According to a first embodiment, the invention relates to a plant or a plant cell comprising a defective PSK function. The term “PSK function” indicates any activity
15 mediated by a PSK peptide or receptor in a plant cell. The PSK function may be effected by the *PSK* gene expression or the PSK peptide activity as well as the *PSKR* gene expression or the PSKR receptor activity.

Within the context of this invention, the terms “defective”, “inactivated” or
20 “inactivation”, in relation to PSK function, indicate a reduction in the level of active PSK peptide or active PSKR receptor present in the cell or plant. Such a reduction is typically of about 20%, more preferably 30%, as compared to a wild-type plant. Reduction may be more substantial (e.g., above 50%, 60%, 70%, 80% or more), or complete (i.e., knock-out plants).

25 Inactivation of PSK or its receptor may be carried out by techniques known per se in the art such as, without limitation, by genetic means, enzymatic techniques, chemical methods, or combinations thereof. Inactivation may be conducted at the level of DNA, mRNA or protein, and inhibit the expression (e.g., transcription or translation) or the
30 activity of PSK or PSKR.

Preferred inactivation methods affect expression and lead to the absence of production of a functional PSK peptide and/or PSKR receptor in the cells. It should be noted that the inhibition of PSK or PSKR may be transient or permanent.

- 5 In a first embodiment, defective PSK or PSKR is obtained by deletion, mutation, insertion and/or substitution of one or more nucleotides in one or more *PSK* or *PSKR* gene(s). In a preferred embodiment, all the *PSK* genes are inactivated in the plant of interest. This may be performed by techniques known *per se* in the art, such as e.g., site-specific mutagenesis, ethyl methanesulfonate (EMS) mutagenesis, targeting induced
10 local lesions in genomes (TILLING), EcoTILLING, homologous recombination, conjugation, etc.

The TILLING approach according to the invention aims to identify SNPs (single nucleotide polymorphisms) and/or insertions and/or deletions in a *PSK* or *PSKR* gene
15 from a mutagenized population. It can provide an allelic series of silent, missense, nonsense, and splice site mutations to examine the effect of various mutations in a gene. EcoTILLING is a variant of TILLING, which examines natural genetic variation in populations.

- 20 Another particular approach is gene inactivation by insertion of a foreign sequence, e.g., through transposon mutagenesis using mobile genetic elements called transposons, which may be of natural or artificial origin.

In the most preferred embodiment, the defective PSK or PSKR is obtained by knock-out
25 techniques, e.g., deletion of all or a portion of the gene, the deleted portion having a size sufficient to prevent expression of a functional protein from the gene. The deleted portion preferably comprises at least 50 consecutive nucleotides of the gene. In a particular embodiment, the deleted gene or portion is replaced in the genome by an inserted foreign nucleic acid.

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According to another preferred embodiment, the defective PSK or PSKR is obtained by gene silencing using RNA interference, ribozyme or antisense technologies. In a

particular embodiment, an inhibitory nucleic acid molecule which is used for gene silencing comprises a sequence that is complementary to a sequence common to several PSK or PSKR genes or RNAs. Preferably, such an inhibitory nucleic acid molecule comprises a sequence that is complementary to a sequence present in all PSK genes or
 5 RNAs or PSKR genes or RNAs of a same species, e.g., *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Triticum aestivum*, *Asparagus officinalis*, *Brassica napus*, *Beta vulgaris*, *Solanum tuberosum*, *Glycine max*, *Vitis vinifera* and/or *Daucus carota*.

10 PSK or PSKR synthesis in a plant may also be reduced by mutating or silencing genes involved in the PSK or PSKR biosynthesis pathway, e.g. those encoding sulfotransferases (SOTs) required for sulfation of the PSK tyrosine residues. Alternatively, PSK or PSKR synthesis and/or activity may also be manipulated by (over)expressing negative regulators of PSK or PSKR, such as transcription factors or
 15 second messengers. In another embodiment, a mutant allele of a gene involved in PSK or PSKR synthesis may be (over)expressed in a plant.

PSK or PSKR inactivation may also be performed transiently, e.g., by applying (e.g., spraying) an exogenous agent to the plant, for example molecules that inhibit PSK or
 20 PSKR activity.

Preferred inactivation is a permanent inactivation produced by destruction of the integrity of the *PSK* or *PSKR* genes, e.g., by deletion of a fragment (e.g., at least 50 consecutive bp) of the gene sequence and/or by insertion of a foreign sequence. As
 25 illustrated in the examples, *psk* or *pskr* knock-out plants with a defective *PSK* or *PSKR* gene are still viable, show no aberrant developmental phenotype, and exhibit increased resistance to plant pathogens.

In a specific embodiment, more than one *PSK* or *PSKR* gene(s) are rendered defective
 30 by knock-out techniques.

In another embodiment, defective PSK function is obtained at the level of the PSK peptide. For example, the PSK peptide may be inactivated by exposing the plant to, or by expressing in the plant cells an antibody directed against the PSK peptide (e.g., anti-sulfotyrosine monoclonal antibody).

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The PSK peptide may also be inactivated by exposing the plant to, or by overexpressing PSKR containing an extracellular binding domain but devoid of the intracellular signaling domain.

10 Alternatively, defective PSK function is obtained by alteration of the PSKR receptor activity. More specifically, the PSKR receptor may be inactivated by antagonists of the PSKR receptor. In a particular embodiment, such antagonists bind to the residues of Glu503-Lys517 of the PSKR receptor.

15 Thus, the PSK function in plant resistance may be controlled at the level of *PSK* genomic DNA, *PSK* mRNA, *PSK* peptide, *PSKR* genomic DNA, *PSKR* mRNA, or *PSKR* receptor activity.

In a variant, the invention relates to a plant with increased resistance to plant pathogens,
20 wherein said plant comprises an inactivated *PSK* gene, more specifically an inactivated *PSK* genomic DNA. The defective *PSK* gene is preferably selected from *PSK 1*, *PSK 2*, *PSK 3*, *PSK 4* and *PSK 5*. In another preferred embodiment, all the *PSK* genes present in the plant are defective, for example all of *PSK1-5* genes.

25 In another variant, the invention relates to a plant with increased resistance to plant pathogens, wherein said plant comprises an inactivated PSK peptide.

In another variant, the invention relates to a plant with increased resistance to plant pathogens, wherein said increased resistance is due to inactivation of a *PSKR* genomic
30 DNA. The defective *PSKR* gene may be the ortholog of the *Arabidopsis PSKR1* gene.

In another variant, the invention relates to a plant with increased resistance to plant pathogens, wherein said increased resistance is due to inactivation of a PSK or PSKR mRNA.

- 5 In another embodiment, the invention relates to transgenic plants or plant cells which have been engineered to be (more) resistant to plant pathogens by inactivation of PSK function. In a particular embodiment, the modified plant is a loss-of-function *psk* or *pskr* mutant plant, with increased resistance to plant pathogens.
- 10 The invention also relates to seeds of plants of the invention, as well as to plants, or descendents of plants grown or otherwise derived from said seeds, said plants having an increased resistance to pathogens.

The invention also relates to vegetal material of a plant of the invention, such as roots,
15 leaves, flowers, callus, etc.

The invention also provides a method for producing plants having increased resistance to pathogens, wherein the method comprises the following steps:

- (a) inactivation of *PSK* and/or *PSKR* gene(s) in a plant cell;
- 20 (b) optionally, selection of plant cells of step (a) with defective *PSK* and/or *PSKR* gene(s);
- (c) regeneration of plants from cells of step (a) or (b); and
- (d) optionally, selection of a plant with increased resistance to pathogens, said
plant with increased resistance to pathogens having defective *PSK* or *PSKR*
25 gene(s).

Inactivation of the *PSK* and/or *PSKR* gene can be done as disclosed above. Genetic alteration in the *PSK* or *PSKR* gene may also be performed by transformation using the Ti plasmid and *Agrobacterium* infection method, according to the protocol described
30 e.g., by Toki *et al* (2006). In a preferred method, inactivation is caused by *PSK* or *PSKR* gene destruction using e.g., knock-out techniques.

Selection of plant cells having a defective *PSK* and/or *PSKR* gene can be made by techniques known *per se* to the skilled person (e.g., PCR, hybridization, use of a selectable marker gene, protein dosing, western blot, etc.).

- 5 Plant generation from the modified cells can be obtained using methods known *per se* to the skilled worker. In particular, it is possible to induce, from callus cultures or other undifferentiated cell biomasses, the formation of shoots and roots. The plantlets thus obtained can be planted out and used for cultivation. Methods for regenerating plants from cells are described, for example, by Fennell *et al.* (1992) Plant Cell Rep. 11: 567-
10 570; Stoeger *et al* (1995) Plant Cell Rep. 14: 273-278.

The resulting plants can be bred and hybridized according to techniques known in the art. Preferably, two or more generations should be grown in order to ensure that the genotype or phenotype is stable and hereditary.

15

Selection of plants having an increased resistance to a pathogen can be done by applying the pathogen to the plant, determining resistance and comparing to a *wt* plant.

- Within the context of this invention, the term “increased resistance” to pathogen means
20 a resistance superior to that of a control plant such as a wild type plant, to which the method of the invention has not been applied. The “increased resistance” also designates a reduced, weakened or prevented manifestation of the disease symptoms provoked by a pathogen. The disease symptoms preferably comprise symptoms which directly or indirectly lead to an adverse effect on the quality of the plant, the quantity of
25 the yield, its use for feeding, sowing, growing, harvesting, etc. Such symptoms include for example infection and lesion of a plant or of a part thereof (e.g., different tissues, leaves, flowers, fruits, seeds, roots, shoots), development of pustules and spore beds on the surface of the infected tissue, maceration of the tissue, accumulation of mycotoxins, necroses of the tissue, sporulating lesions of the tissue, colored spots, etc. Preferably,
30 according to the invention, the disease symptoms are reduced by at least 5% or 10% or 15%, more preferably by at least 20% or 30% or 40%, particularly preferably by 50% or

60%, most preferably by 70% or 80% or 90% or more, in comparison with the control plant.

The term “increased resistance” of a plant to pathogens also designates a reduced susceptibility of the plant towards infection with plant pathogens or lack of such susceptibility. The inventors have demonstrated, for the first time, a correlation between expression of *PSK* or *PSKR* genes and susceptibility towards infection. As shown in the experimental part, infection of plants with oomycete pathogens, triggers transcriptional activation of *PSK* and *PSKR1* genes. Furthermore, the inventors have shown that the overexpression of *PSK* genes and of *PSKR1* promotes disease, whereas the knockout of *PSK3* and of *PSKR1* increases resistance. The inventors have therefore proposed that the *PSK* signaling increases susceptibility of plants to infection and favors the development of the disease. Thus, in a preferred embodiment, the resistance of *PSK*- or *PSKR*-defective plants to plant pathogens is due to a loss of susceptibility of these plants to pathogens.

Preferred plants or cells of the invention should be homozygous with respect to *PSK* or *PSKR* gene inactivation, i.e., both *PSK* or *PSKR* alleles are inactive.

In the most preferred embodiment, the method of the invention is used to produce dicot or monocot plants having a defective *PSK* or *PSKR* gene with increased resistance to oomycete, nematode and/or bacterial pathogens. Examples of such plants and their capacity to resist pathogens are disclosed in the experimental section.

A particular object of the invention relates to a *Solanaceae* plant, preferably a tomato plant, wherein the cells of said plant lack all or part of a *PSK* or *PSKR1* gene and are defective for *PSK* function. Such plants exhibit increased resistance to pathogens such as fungus, oomycetes, nematodes or bacterial pathogens. In a preferred embodiment, the invention relates to a tomato plant wherein the cells of said plant lack all or part of the *PSKR1* gene. A preferred plant lacks at least a portion (i.e., more than 50 consecutive nucleotides) of the gene within target1 or target2 as disclosed Fig.13. Even more

preferably, the deleted portion encompasses at least one of the following nucleotides: A88, T119, G502, G856, G2285 and G1978.

Another particular object of the invention relates to a *Solanaceae* plant, preferably a tomato plant, wherein the cells of said plant have a mutated *PSKR1* gene and are defective for PSK function. Such plants exhibit increased resistance to pathogens such as fungus, oomycetes, nematodes or bacterial pathogens. In a preferred embodiment, the mutation is present in target1 or target2 domains as disclosed Fig 13. Even more preferably, the mutation is selected from pskr1.1 A88 T, pskr1.2 T119 C, pskr1.3 G502 A, pskr1.4 G856 A, pskr1.5 G2285 A and pskr1.6 G1978 A.

Another particular object of the invention relates to a *Apiaceae* plant, preferably a carrot plant, wherein the cells of said plant lack all or part of a *PSK* or *PSKR1* gene and are defective for PSK function. Such plants exhibit increased resistance to pathogens such as fungus, oomycetes, nematodes or bacterial pathogens.

Another particular object of the invention relates to a *Poaceae* plant, preferably a wheat, rice, barley, oat, rye, sorghum or maize plant, wherein the cells of said plant lack all or part of a *PSK* or *PSKR1* gene and are defective for PSK function. Such plants exhibit increased resistance to pathogens such as fungus, oomycetes, nematodes or bacterial pathogens.

Screening of plant resistance modulators

The invention also discloses novel methods of selecting or producing regulators of plant resistance, as well as tools and constructs for use in such methods.

In a particular aspect, the invention relates to a method for screening or identifying a molecule that modulates plant resistance, the method comprising testing whether a candidate compound modulates *PSKR* gene expression or activity. The test can be performed in a cell containing a reporter DNA construct cloned under control of *PSKR* promoter sequence, or in a cell expressing *PSKR* or *PSKR* fusion protein.

Preferably, such a method comprises the following steps:

- providing a cell comprising a nucleic acid construct that comprises the sequence of a *PSKR* gene promoter operably linked to a reporter gene;
 - 5 - contacting the cell with a candidate molecule;
 - measuring the activity of *PSKR* promoter by monitoring of the expression of a marker protein encoded by the reporter gene in the cell; and
 - selecting a molecule that modulates the expression of the marker protein.
- 10 In another embodiment, the invention also relates to methods for screening or identifying a molecule that modulates the *PSKR* activity, comprising the following steps:
- providing a cell comprising a reporter gene under the control of a transcription factor, and a fusion protein comprising a *PSKR* protein fused to the DNA binding domain of
 - 15 the transcription factor;
 - contacting said cell with another fusion protein comprising a candidate molecule fused to the transcriptional activation domain of the transcription factor;
 - measuring the activity of the *PSKR* by monitoring of the expression of a marker protein encoded by the reporter gene in the cell, said marker protein being expressed
 - 20 only if both fusion proteins are interacting;
 - selecting a molecule that induces the expression of the marker protein.

Preferred modulators are inhibitors of the expression of *PSKR*.

- 25 In a further embodiment, the invention also relates to the use of compounds that inhibit *PSKR* expression or activity for increasing resistance of plants to plant pathogens. Such compounds are typically identified using the above method of screening. The use of such compounds typically comprise exposing a plant to such compound, e.g., by spraying or in a mixture with water, thereby causing transient *PSK* inactivation, and
- 30 transient increase in resistance to pathogens.

In this regard, the invention also relates to an antibody that specifically binds a PSK peptide or receptor, or a fragment or derivative of such antibody having essentially the same antigenic specificity. Such an antibody may be polyclonal or, more preferably, monoclonal. Examples of antibody fragments include Fab fragment, Fab' fragment, 5 CDR domains. Examples of derivatives include single chain antibodies, humanized antibodies, recombinant antibodies, etc. Such antibodies may be produced by techniques known *per se* in the art, such as immunization and isolation of polyclonal antibodies or, immunization, isolation of antibody-producing cells, selection and fusion thereof with e.g., myeloma cells, to produce hybridoma producing monoclonal antibodies. 10 Fragments and derivatives thereof may be prepared using known techniques. An antibody specific for a PSK peptide or receptor is an antibody that binds such a peptide or receptor with a higher affinity than other peptides or receptors. Preferred specific antibodies essentially do not bind other peptides or receptors.

15 In another embodiment, the invention also relates to methods for identifying proteins, which interact with PSKR, which are required for functional PSKR signaling, and which might be additional targets for inactivation to increase resistance. Such screening methods are preferentially Y2H systems that allow identifying interaction partners of cytoplasmic and membrane-bound proteins, such as the split-ubiquitin system (Stagljar 20 *et al.*, 1998), and the mating-based split-ubiquitin system (Grefen *et al.*, 2009). Proteins interacting with individual PSKR domains might also be identified with the classical GAL4 Y2H system that works in the yeast nucleus (Fields and Song, 1989).

Further aspects and advantages of the invention are provided in the following examples, 25 which are given for purposes of illustration and not by way of limitation.

EXAMPLES

MATERIALS AND METHODS

Generation of mutant and transgenic *Arabidopsis* lines for the functional analysis of genes encoding the phytosulfokines PSK1, PSK2, PSK3, PSK4, PSK5 and their receptor PSKR1.

Several mutant and transgenic lines listed in Table 1 have been analyzed by the
5 inventors.

Table 1: Mutant and transgenic *Arabidopsis* lines

AGI	Gene	FST	Clone name	Line	Amplification attB1-attB2	Vector	Ecotype
At2g02220	<i>PSKR1</i>	SAIL_245_H03.V1	<i>pskr1-1</i>	Mutant			Col N8846
At2g02220	<i>PSKR1</i>	407D02	<i>pskr1-2</i>	Mutant			Ws
At2g02220	<i>PSKR1</i>	308B10	<i>pskr1-3</i>	Mutant			Col-0
At2g02220	<i>PSKR1</i>	SALK-008585	<i>pskr1-4</i>	Mutant			Col-0 CS60000
At2g02220	<i>PSKR1</i>		<i>Cpskr1-2</i>	<i>pskr1-2</i> complementation	5472 bp	pHGW (Karimi et al., 2002)	Ws
At2g02220	<i>PSKR1</i>		p35s:PSKR1	PSKR1 overexpression	3060 bp	pH2GW7 (Karimi et al., 2002)	Ws
At2g02220	<i>PSKR1</i>		p35s:PSKR1:GFP	PSKR1 overexpression with C-terminal GFP	3056 bp	pK7FWG2.0 (Karimi et al., 2002)	Ws
At2g02220	<i>PSKR1</i>		PSKR1pro:GFP:GUS	PSKR1 expression analysis	1795 bp	pKGWFS7 (Karimi et al., 2002)	Ws
At1g13590	<i>PSK1</i>	SALK_036304	<i>psk1-1</i>	Mutant			Col-0 CS60000
At2g22860	<i>PSK2</i>		p35s:PSK2	PSK2 overexpression	294 bp	pK2GW7 (Karimi et al., 2002)	Ws
			PSK2pro:GFP:GUS	PSK2 expression analysis	1005 bp	pKGWFS7 (Karimi et al., 2002)	Ws
			p35s:PSK2:GFP	PSK2 overexpression with C-terminal GFP	291 bp	pK7FWG2.0 (Karimi et al., 2002)	Col-0
			PSK2-RNAi	PSK2-RNAi	291 bp	pH7GWIWG2(II) (Karimi et al., 2002)	Ws
At3g44735	<i>PSK3</i>	SAIL_378_F03	<i>psk3-1</i>	Mutant			Col N8846
At3g49780	<i>PSK4</i>		p35s:PSK4	PSK4 overexpression	282 bp	pK2GW7 (Karimi et al., 2002)	Ws
At5g65870	<i>PSK5</i>	SALK_043834	<i>psk5-1</i>	Mutant			Col-0 CS60000
	<i>PSKα</i>		p35S:spPSK4-pepPSK	PSK α overexpression	135bp	pK2GW7 (Karimi et al., 2002)	Ws
	<i>PSKα</i>		p35S:spPSK4-pepPSK- HA	PSK α overexpression with C-terminal HA tag	228bp	pK2GW7 (Karimi et al., 2002)	Ws

For **p35s:PSK2**, a fragment of 294 bp of entire coding sequence was amplified by PCR
using the primers attB1 (5'-
10 AAAAAGCAGGCTTCACCATGGCAAACGTCTCCGCTTTGC-3'; SEQ ID NO: 41)
and attB2 (5'-AGAAAGCTGGGTGTCAAGGATGCTTCTTCTTCTGG-3'; SEQ ID
NO: 42). The PCR fragment was inserted into the pDON207 donor vector and then in
the plant expression vector pK2GW7 (Karimi et al., 2002) using Gateway technology
(Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-
15 type by *Agrobacterium*-mediated transformation.

For **PSK2pro:GFP:GUS** fusion, a fragment of 1005 bp upstream of the start codon was
amplified by PCR using the primers attB1 5'-
AAAAAGCAGGCTTCTGAAGTTTGGTGCATTAATT-3'; SEQ ID NO: 43) and
attB2 (5'-AGAAAGCTGGGTGTTTTGTGATATTTCTTTGAAG-3'; SEQ ID NO:
20 44). The PCR fragment was inserted into the pDON207 donor vector and then in the
plant expression vector pKGWFS7 (Karimi et al., 2002) using Gateway technology
(Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-

type by *Agrobacterium*-mediated transformation. Using the PSK2pro:GFP:GUS construction, the inventors have demonstrated that PSK2 gene is developmentally regulated (Figure 1).

For **p35s:PSK2:GFP** fusion and **PSK2-RNAi**, a fragment of 291 bp of entire coding sequence without stop codon was amplified by PCR using the primers attB1 (5'-
 5 AAAAAGCAGGCTTCACCATGGCAAACGTCTCCGCTTTGC-3'; SEQ ID NO: 45) and attB2 (5'-AGAAAGCTGGGTGAGGATGCTTCTTCTTCTGG-3'; SEQ ID NO: 46). The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pK7FWG2,0 (Karimi *et al.*, 2002) for p35s:PSK2:GFP or
 10 pH7GWIWG2(II) (Karimi *et al.*, 2002) for PSK2-RNAi using Gateway technology (Invitrogen). The T-DNAs from the resulting vectors were transferred into Col and Ws wild-types, respectively, by *Agrobacterium*-mediated transformation.

For **p35s:PSK4**, a fragment of 282 bp of entire coding sequence was amplified by PCR using the primers attB1 (5'-
 15 AAAAAGCAGGCTTCACCATGGGTAAGTTCACAACCATTT-3'; SEQ ID NO: 47) and attB2 (5'-AGAAAGCTGGGTGTCCACCTCCGGATCAGGGCTTGTGATTCTGAGTA-3'; SEQ ID NO: 48). The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pK2GW7 (Karimi *et al.*, 2002) using Gateway
 20 technology (Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-type by *Agrobacterium*-mediated transformation.

The transgenic line **spPSK4-pepPSK** was generated to constitutively express a fusion between the PSK4 signal sequence for secretion and the PSK α minimal motif. A fragment of 113 bp comprising the fusion was obtained by annealing the two primers,
 25 forPSK4PS-PSK (5'-AATTCATGGGTAAGTTCACAACCATTTTCATCATGGCTCTCCTTCTTTGCTCTA
CGCTAACCTACGCAGAAGAGTTTCATACGGACTACATCTACACTCAGGACGT
AA-3'; SEQ ID NO: 49) and revPSK4PS-PSK (5'-AGCTTTACGTCCTGAGTGTAGATGTAGTCCGTATGAACTCTTCTGCGTAGGT
 30 TAGCGTAGAGCAAAGAAGGAGAGCCATGATGAAAATGGTTGTGAACTTACC
CATG-3'; SEQ ID NO: 50). This fragment was ligated into EcoRI/HindIII- digested

pBlueScript. A 135 bp PCR fragment obtained from this vector as a template using the primers attB1 forPSK-B1 (5'-AAAAAGCAGGCTTCATGGGTAAGTTCACAACC-3'; SEQ ID NO: 51) and attB2 revPSKstop-B2 (5'-AGAAAGCTGGGTATCACTTTACGTCCTGAGTGTAG -3'; SEQ ID NO: 52) was then inserted into the pDON207 donor vector and then in the plant expression vector pK2GW7 (Karimi *et al.*, 2002) using Gateway technology (Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-type by *Agrobacterium*-mediated transformation.

The transgenic line **spPSK4-pepPSK-HA** was generated to constitutively express a fusion between the PSK4 signal sequence for secretion and the PSK α minimal motif harboring a C-terminal HA tag. A fragment of 113 bp was obtained by annealing of the two primers, forPSK4PS-PSK (5'-AATTCATGGGTAAGTTCACAACCATTTTCATCATGGCTCTCCTTCTTTGCTCTACGCTAACCTACGCAGAAGAGTTTCATACGGACTACATCTACACTCAGGACGTAA-3'; SEQ ID NO: 53) and revPSK4PS-PSK (5'-AGCTTTACGTCCTGAGTGTAGATGTAGTCCGTATGAAACTCTTCTGCGTAGGTAGCGTAGAGCAAAGAAGGAGAGCCATGATGAAAATGGTTGTGAACTTACC CATG -3'; SEQ ID NO: 54). This fragment was ligated into *EcoRI/HindIII*- digested pBlueScript. For 3HA-tag insertion, a fragment of 111 bp was amplified by PCR using the primers forHA-Hind (5'-GGTAAGCTTTACCCATACGATGTTCCCTG-3'; SEQ ID NO: 55) and revHA-XhoI (5'-GAACTCGAGTCAAGCGTAATCTGGAACGTC-3'; SEQ ID NO: 56) on pNX32-Dest with following digestion by HindIII/XhoI. Digested 3HA-tag fragment was ligated into HindIII/XhoI - digested pBlueScript containing the fusion between the PSK4 signal sequence and the PSK α minimal sequence (without stop codon). A fragment of 228 bp was amplified by PCR using the primers attB1 forPSK-B1 (5'-AAAAAGCAGGCTTCATGGGTAAGTTCACAACC-3'; SEQ ID NO: 57) and attB2 revPSK-HAstop-B2 (5'-AGAAAGCTGGGTGTCAAGCGTAATCTGGAACG - 3'; SEQ ID NO: 58). The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pK2GW7 (Karimi *et al.*, 2002) using Gateway technology (Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-type by *Agrobacterium*-mediated transformation.

For **Cp ψ skr1-2**, a fragment of 5472 bp including 1771 bp upstream of the start codon (promoter and 5'UTR), 3027 bp of entire coding sequence and 650 bp of 3' non coding sequence (3'UTR and terminator) was amplified by PCR using the primers attB1 (5'-AAAAAGCAGGCTTCATGGCAAGAAAATGTGAGAC-3'; SEQ ID NO: 59) and
 5 attB2 (5'-AGAAAGCTGGGTGGAACCATTATAGGAAGCGTACTAATC-3'; SEQ ID NO: 60). The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pHGW (Karimi *et al.*, 2002) using Gateway technology (Invitrogen). The T-DNA from the resulting plant expression vector was transferred into the *pskr1-2* mutant by *Agrobacterium*-mediated transformation.

10 For **p35s:PSKR1 (PSKR1-OE)**, a fragment of 3060 bp of the entire coding sequence was amplified by PCR using the primers attB1 (5'-AAAAAGCAGGCTGTTCTTGAAATGCGTGTTTCATCG-3'; SEQ ID NO: 61) and attB2 (5'-AGAAAGCTGGGTCTAGACATCATCAAGCCAAGAGAC-3'; SEQ ID NO: 62). The PCR fragment was inserted into the pDON207 donor vector and then in
 15 the plant expression vector pH2GW7 (Karimi *et al.*, 2002) using Gateway technology (Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-type by *Agrobacterium*-mediated transformation.

For **p35s:PSKR1:GFP** fusion, a fragment of 3056 bp of entire coding sequence without stop codon was amplified by PCR using the primers attB1 (5'-AAAAAGCAGGCTTTACCATGCGTGTTTCATCGTTTTT-3'; SEQ ID NO: 63) and
 20 attB2 (5'-AGAAAGCTGGGTAGACATCATCAAGCCAAGAGACT-3'; SEQ ID NO: 64). The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pK7FWG2.0 (Karimi *et al.*, 2002) using Gateway technology (Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-
 25 type by *Agrobacterium*-mediated transformation.

For **PSKR1pro:GFP:GUS** fusion, a fragment of 1795 bp upstream of the start codon was amplified by PCR using the primers attB1 5'-AAAAAGCAGGCTTCATGGCAAGAAAATGTGAGAC-3'; SEQ ID NO: 65) and
 attB2 (5'-AGAAAGCTGGGTTCACGAACAGAGGAAGAAG-3'; SEQ ID NO: 66).
 30 The PCR fragment was inserted into the pDON207 donor vector and then in the plant expression vector pKGWFS7 (Karimi *et al.*, 2002) using Gateway technology

(Invitrogen). The T-DNA from the resulting vector was transferred into the Ws wild-type by *Agrobacterium*-mediated transformation. Using the PSKR1pro:GFP:GUS construction, the inventors have demonstrated that PSKR1 gene is developmentally regulated (Figure 3).

5 **Example 1: PSK mutants are more resistant to infection by *M. incognita* and *H. arabidopsidis*.**

I) PSK expression during plant development:

Expression of the *PSK2* gene during root and leaf development was analyzed through reporter gene activities in the transgenic line PSK2pro:GFP:GUS.

10 Results:

As shown in Figure 1, expression of the *PSK2* gene is developmentally regulated. GUS activity (A) and GFP (B) revealing *PSK2* promoter activation is detectable in the root tips (lateral root cap) but not in the elongation zone. In fully differentiated roots (C,D), *PSK2* expression localizes to the vascular cylinder, and to the lateral root primordia (E).
15 *PSK2* expression in the shoots is localized in the vascular system of leaves and cotyledons (F), trichomes (G), and stomata (H,I).

II) Gene expression analysis of response to pathogens using microarray:

Expression profiling of *PSK* genes during the compatible interaction with *M. incognita* and *H. arabidopsidis* was analyzed by microarray hybridizations. Samples were
20 prepared from isolated galls and infected cotyledons at different time points after infection with *M. incognita* and *H. arabidopsidis*, respectively. Sample preparations, hybridizations on CATMA (*M. incognita*) and Affymetrix ATH1 (*H. arabidopsidis*) microarrays, and data analyses were performed as described (Jammes *et al.*, 2005; Hok *et al.*, 2011).

25 Results:

As shown in Figure 2A, only genes encoding *PSK2* and *PSK4* are represented on CATMA arrays, and were downregulated at all stages of developing galls. The same genes were upregulated in infected cotyledons, particularly at late stages of downy mildew infection. Additionally, an upregulation of the gene encoding *PSK5* is observed,

whereas genes encoding PSK1 and PSK3 do not change (nc) expression intensities upon infection with *H. arabidopsidis*.

III) Gene expression analysis of response to pathogens using real time quantitative RT-PCR

5 Relative *PSK* transcript accumulations in *Arabidopsis* galls were measured at 7 (white bars), 14 (grey bars), and 21 (black bars) days after nematode inoculation (DAI) by quantitative RT-PCR in comparison to uninfected roots. The *PSK* expression ratio was established with the $2^{-(\Delta\Delta C_t)}$ method, comparing the ΔC_t for the gene of interest (C_t uninfected - C_t infected) with the ΔC_t for the reference gene (C_t uninfected - C_t infected), where the gene of interest is one of the analyzed *Arabidopsis PSK* genes (*PSK1-PSK5*) and the reference gene is *AtUBP22* (At5g10790). A ratio equaling 1 indicates that the *PSK* gene is not regulated by nematode infection. A ratio < -1 and > 1 indicate gene repression and activation, respectively. Two biological replicates were performed. The results are shown in Figure 2B.

15 IV) Gene expression analysis of response to pathogens using reporter gene expression

PSK2 expression pattern was analyzed in galls of *M. incognita*-infected roots of the *Arabidopsis PSK2pro:GFP:GUS* reporter line as shown in Figure 2C. Images A and B of Figure 2C show a reduced GUS activity which is revealed in the center of galls forming at 5 (A) and 14 (B) days after inoculation. Image C of Figure 2C shows projections of serial confocal optical *in vivo* sections show a downregulation of GFP accumulation representing *PSK2* expression in giant cells.

V) Quantitative analysis for the interaction phenotype of *PSK* knock-out mutants

25 Quantitative analysis for the interaction phenotype of the *psk3* knockout mutant (Figure 6A) with *H. arabidopsidis* was carried out (Figure 6B). Seeds from the different *A. thaliana* lines were sown on a soil/sand mixture, stratified for 3 days at 4 °C, and then grown under a 12 h photoperiod in a growth chamber at 20 °C. The *H. arabidopsidis* isolate, Emwa1 and Noco2 were transferred weekly onto the susceptible accession Ws-0 and Col-0, respectively, as described previously (Dangl *et al.*, 1992). For infection, 10-

day-old plants were spray-inoculated to saturation with a spore suspension of 40,000 spores/ml of the virulent isolate Noco2. Plants were kept in a growth cabinet at 16°C for 6 d with a 12 h photoperiod. Sporulation was induced by spraying plants with water, and keeping them for 24 h under high humidity. Plantlets were collected 7 days post inoculation in 1 ml of water, vortexed, and the titer of liberated conidiospores was determined with a hemocytometer. Sporulation of *H. arabidopsidis* isolate Noco2 on cotyledons of the *Arabidopsis psk3-1* mutant was reduced by >50 %, when compared to wild-type plants (Col-0). Plantlets were collected 7 days post inoculation in 1 ml of water, vortexed, and the titer of liberated conidiospores was determined with a hemocytometer. For statistics, 20 samples at 10 plantlets were prepared for each line and analysis. The experiment was repeated 3 times with similar results. Statistically significant differences for values compared with the wild type were determined by Student's t-test (*** P<0.0001).

Example 2: *Pskr1* knock-out mutants are less susceptible to *H. arabidopsidis*.

Molecular analyses of 4 allelic *Arabidopsis pskr1* knockout mutants have been conducted. The mutant lines *pskr1-1* (SAIL_245_H03), *pskr1-2* (FLAG_407D02), *pskr1-3* (GABI_308B10), and *pskr1-4* (SALK-008585) were from the Syngenta *Arabidopsis* Insertion Library, from INRA (Versailles, France), from the Max-Planck-Institut (Cologne, Germany), and from the SALK Institute (LaJolla, USA), respectively. All lines are publicly available and were obtained from the Nottingham *Arabidopsis* Stock Center (*pskr1-1*, *pskr1-3*, and *pskr1-4*) and INRA Versailles (*pskr1-2*).

Primer attachment sites, and T-DNA insertion sites and orientations in the genome are indicated in Figure 8A.

RT-PCR revealed *PSKR1* transcripts in wild-type *Arabidopsis* (Col-N8846, Ws, Col-0, and Col-8 CS60000) as shown in Figure 8B. Amplicons spanning the insertion sites were absent from all allelic mutants. Amplicons revealing transcripts with primers 3' of the insertion sites most likely originate from transcriptional initiation within the T-DNA, as previously reported for other insertion lines (Chinchilla *et al.*, 2007, *Nature* 448, 497-500). Amplification of transcripts from the constitutively expressed *AtEF1α* gene

(At1g07930) showed that similar amounts of intact cDNAs were used for RT-PCR experiments.

For infection, 10-day-old plants were spray-inoculated to saturation with a spore suspension of 40,000 spores/ml of the virulent isolate (Emwa1 on the Ws wild-type and *pskr1-2*, Noco2 on the other wild-types and mutants). For statistical analysis of sporulation, 20 samples at 10 plantlets were prepared for each line and analysis. The bars represent mean values ($\pm$ SD), and *** indicates significant differences between wild-type and mutant lines with $P < 0.0001$, as determined by Student's t-test. All experiments were repeated 3 times and gave similar results. 1-1, 1-2, 1-3, and 1-4 represent the mutants *pskr1-1*, *pskr1-2*, *pskr1-3*, and *pskr1-4*, respectively.

Results:

As shown in Figure 8C, all allelic *pskr1* knock-out mutants exhibit an increased downy mildew resistance. Asexual reproduction, an indicator for disease provoked by the downy mildew oomycete pathogen, is reduced by $> 50\%$.

15

Example 3: *Pskr1* knock-out mutants are less susceptible to *M. incognita*.

Arabidopsis plants were infected *in vitro* 14 days after germination with 150 surface-sterilized freshly hatched *M. incognita* J2. Infected seedlings were kept at 20°C with a 16-h photoperiod. During infection tests, egg mass counting was performed 60 DAI (days after inoculation) to allow nematodes to complete their life cycle. The nematode infects roots and initiates gall formation to a similar extent in *pskr1* mutants and wild-type plants, as analyzed 10 days post inoculation (Dpi). A reduction in the amount of mature galls is observed in *pskr1* mutants at 21 Dpi. The inhibition of nematode development in the absence of PSKR1 becomes most evident during the parthenogenetic production of egg masses, which are strongly reduced on *pskr1* mutants at 75 Dpi.

25

Results:

As shown in Figure 9, allelic *pskr1* mutants are less susceptible to *M. incognita* since root knot nematode reproduction is strongly inhibited in the absence of PSKR1. The

30

production of galls and egg masses, which are indicators for disease provoked by the root knot nematode, is strongly reduced.

Example 4: The *pskr1* knock-out mutants are less susceptible to infection by *R. solanacearum*.

A. thaliana seeds were sterilized for 20 min with a 12% sodium hypochlorite solution, washed several times with sterile water and sown on MS medium. Plantlets grown for 8 days at 20°C in a growth chamber were then transferred to Jiffy pots (Jiffy France, Lyon, France) and grown for 3 weeks in short day conditions (10 h light at 500 $\mu\text{E s}^{-1}\text{m}^{-2}$). Plants with a Ws and Col genetic background (mutant plants, complemented mutant plants, and plants overexpressing *PSKR1*) were root-inoculated with the virulent bacterial isolates RD15 and GMI1000, respectively. Approximately 2 cm were cut from the bottom of the Jiffy pots and the exposed roots of the plants were immersed for 3 min in a suspension containing 10^7 bacteria per ml. The plants were then transferred to a growth chamber with a day/night cycle of 8 h at 27°C, 120-140 $\mu\text{E m}^{-1}\text{s}^{-2}$ and 16 h at 26°C, respectively, keeping relative humidity at 75 %. Disease symptoms on inoculated plants were scored at 3, 4, 5, 6, and 7 days post inoculation according to a disease index (DI) covering DI 0 (no wilt), and DI 1, DI 2, DI 3, and DI 4, representing 25 %, 50 %, 75 %, and 100% of wilted leaves.

Results:

pskr1 knock-out mutants exhibit a reduced susceptibility to the bacterial pathogen *Ralstonia solanacearum* since the appearance of bacterial wilt symptoms was delayed in the absence of *PSKR1* (Figure 10). The observed enhanced resistance during the exponential bacterial growth phase between 3 and 5 days post inoculation was significant, with $P < 0.0001$. The Col genetic background (Figure 10B) of *A. thaliana* showed an overall higher susceptibility to *R. solanacearum*, and the effect of the *pskr1* mutation is most pronounced in *pskr1-2* in the Ws genetic background (Figure 10A). Full susceptibility to *R. solanacearum* was restored through the introduction of a functional *PSKR1* gene into the *pskr1-2* genetic background (complemented line *Cppskr1-2*). An acceleration of disease at late time points of infection was observed in

the line overexpressing *PSKR1* under the control of the constitutive 35S promoter (overexpressing line *PSKR1-OE*).

Example 5: *PSKR1* gene expression pattern in *Arabidopsis thaliana* after infection with the downy mildew oomycete pathogen, *H. arabidopsidis*.

PSKR1 transcript abundance was analyzed by qRT-PCR at different time points after spray-treatment of *Arabidopsis* (ecotype Ws-0) cotyledons with water, or with conidiospore suspensions at 40,000 spores/ml of the downy mildew pathogen, *H. arabidopsidis* (Hpa) (see Figure 4A). As shown in Figure 4B, after infection, the expression of *PSKR1* increases continuously and localizes to infected areas of the mesophyll.

Example 6: Infection with the root-knot nematode, *M. incognita*, does not trigger transcriptional activation of the *PSKR1* gene, but downregulates expression in giant cells.

PSKR1 transcript abundance was first analyzed by qRT-PCR at 7, 14 and 21 days after root inoculation. *Arabidopsis* plants were infected *in vitro* 14 d after germination with 150 surface-sterilized freshly hatched *M. incognita* J2 larvae. Infected seedlings were kept at 20°C with a 16-h photoperiod. Relative *PSKR1* mRNA quantities were normalized with *AtUBP22* (At5g10790) using Q-Base. The ratio equals 1 meaning that the *PSKR1* gene is not regulated by nematode infection (Figure 5A). The expression pattern of the GFP reporter gene under control of the *PSKR1* promoter in galls was induced by *M. incognita* in *Arabidopsis* roots, 7 (A) and 21 (B) days after inoculation with 150 surface-sterilized freshly hatched *M. incognita* J2 larvae (Figure 5B). Interestingly, *PSKR1* expression appears being downregulated in giant cells induced by the nematode.

The inventors have hypothesized that PSKR is directly involved in giant cell ontogenesis or may have a role in the cells surrounding the giant cells (where PSKR is expressed) for their divisions or *de novo* formation of vascular elements. The surrounding cells should be also important to obtain functional feeding cells, specialized

sinks that constitute the exclusive source of nutrients for the nematode until reproduction.

Example 7: Plants over-expressing the *PSK* gene are more susceptible to
5 *H. arabidopsidis* and *M. incognita*.

Quantitative analysis for the interaction phenotype with *H. arabidopsidis* of transgenic lines overproducing PSK2 and PSK4 was conducted. Sporulation of *H. arabidopsidis* isolate Emwa1 on cotyledons of the *Arabidopsis* PSK overexpressing lines is strongly increased, when compared to wild-type plants (Ws). For statistics, 20 samples at 10
 10 plantlets were prepared for each line and analysis. The experiment was repeated 3 times with similar results. Statistically significant differences for values compared with the wild type were determined by Student's t-test (*** $P < 0.0001$) as shown in Figure 7A.

Figure 7B shows that root knot nematode reproduction is significantly stimulated in transgenic lines constitutively overexpressing *PSKs*. *Arabidopsis* plants were infected *in*
 15 *vitro* 14 d after germination with 150 surface-sterilized freshly hatched *M. incognita* J2. Infected seedlings were kept at 20°C with a 16-h photoperiod. During infection tests, egg mass counting was performed 75 Dpi (days post inoculation) to allow nematodes to complete their life cycle. The nematode infects roots and initiates gall formation, and develops mature galls to a stronger extent in *PSK* overexpressing plants than in wild-
 20 type plants, as analyzed 10 days and 21 Dpi, respectively. Statistically significant differences were determined by the Student's t test (* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$).

To determine the susceptibility of *PSK* overexpressing lines to *R. solanacearum*, bacterial growth curves were established. Four week-old plants were root-inoculated
 25 with a solution containing 10^7 bacteria per ml of the virulent bacterial isolates RD15. The plants were then transferred to a growth chamber with a day/night cycle of 8 h at 27 °C, 120-140 $\mu\text{E m}^{-1}\text{s}^{-2}$ and 16 h at 26 °C, respectively, keeping relative humidity at 75 %. For establishing bacterial internal growth curves, the aerial parts of three inoculated plants were weighed, sterilized with 250 ml of 70% ethanol for 3 min, rinsed three times
 30 in sterile water, and ground in a mortar after addition of sterile water (2.0 ml per g of fresh weight). Various dilutions of the ground material were then performed with sterile

water and 3 x 40 µl of bacterial suspensions were spotted on petri plates containing solid SMSA medium (Elphinstone *et al.*, 1996), and grown at 30°C. For each time point, triplicate assays were performed for each bacterial strain and *A. thaliana* accession.

Results:

- 5 -Plants overexpressing the *PSK2* or *PSK4* genes are more susceptible to *H. arabidopsidis*. Asexual reproduction, an indicator for disease provoked by the downy mildew oomycete pathogen, is significantly increased in both transgenic lines (Figure 7A).
- 10 -Transgenic plants overexpressing *PSK2* or *PSK4* genes are more susceptible to the nematode pathogen, *M. incognita*. Parthenogenetic production of egg masses at 75 Dpi is significantly enhanced in the transgenic lines, when compared to the wild-type (Figure 7B).
- 15 -Plants overexpressing the *PSK2* or *PSK4* genes are more susceptible to *R. solanacearum*. Figure 7C shows that bacteria multiply faster in transgenic lines over-producing *PSK2*. Multiplication of *R. solanacearum* is strongly increased 3 Dpi, leading to a 100- to 1000-fold higher amount of bacteria in the infected *PSK* overexpressing lines, when compared to wild-type plants (Figure 7C).

Example 8: Plants over-expressing the *PSKR* gene are more susceptible to *H. arabidopsidis*.

- 20 For overexpression of the gene, 3,060 bp of the coding region including Start and Stop codons were amplified from genomic DNA, cloned into the Gateway destination vector pH2GW7 (Karimi *et al.*, 2002), and mobilized into *Arabidopsis* by *Agrobacterium*-mediated transformation. The pathogen assays were performed as described before. All
- 25 experiments were repeated 3 times and gave similar results (see Figure 11A). Relative *PSKR1* transcript accumulations in *Arabidopsis* seedlings (15 days after sowing) were determined by quantitative real time RT-PCR. Expression ratios were calculated using the $2^{-(\Delta\Delta CT)}$ method with *UBP22* (At5g10790) for normalization and wild-type *PSKR1* expression as the reference. The bars ($\pm$ SD) represent mean values of three technical
- 30 replicates (see Figure 11B).

Results:

The *PSKR* expression was analyzed in mutant plants overexpressing *PSKR*. As shown in Figure 11, the overexpression of *PSKR1* (line *PSKR1*-OE) increases downy mildew susceptibility by almost 100 %. Therefore, downy mildew susceptibility correlates with *PSKR1* expression.

Example 9: The increased resistance phenotype of *pskr1* mutants is not due to increased defense mechanisms.

Marker genes for salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene (JA/ethylene)-mediated signaling pathways were *PR1a* (At2g14610) *PDF1.2* (At5g44420), and *PR4* (At3g04720), respectively. Expression of these defense-related genes was analyzed by quantitative real-time RT-PCR in wild type (Ws), mutant (*pskr1-2*), and transgenic *PSKR1* overexpressor (*PSKR1*-OE) plants upon spray treatment of cotyledons with water, or with conidiospore suspensions (40,000 spores/ml) of the *H. arabidopsidis* isolate Emwa1. Samples for RNA extraction and qRT-PCR were prepared at time point 0, and 24, 48, 72, and 120 hours after onset of treatment. Relative quantities of marker gene transcripts were normalized with *AtOXA1* (At5g62050) and *AtUBP22* (At5g10790) using the Q-Base software. Represented are means ($\pm$ SD) from 3 technical replicates. Two independent experiments gave similar results.

Results:

As shown in Figure 12, the activation of SA-, JA-, and JA/ethylene-mediated defense signaling pathways in *Arabidopsis* is independent of *PSKR1*. The *pskr1-2* mutant and *PSKR* overexpressing plants are not altered in these defense signaling pathways, i.e. increased resistance of the *pskr1-2* mutant does not correlate with increased defense, and increased susceptibility of the overexpressing line is not correlated with decreased defense. A rather decreased defense activation in *H. arabidopsidis*-inoculated *pskr1-2* mutant plants reflects most likely reduced downy mildew development.

Exemple 10: *PSKR1* suppression causes reduced pathogen proliferation

Pskr1 mutants were produced as disclosed in Example 4. These plants show delayed disease development in comparison to wild-type plants.

5

In a further set of experiments (see Figure 13), the inventors have investigated whether such reduced susceptibility results from a reduced pathogen proliferation. To that purpose, wild-type plants, *pskr1* mutants, the overexpressor line, and the complemented line were submitted to inoculations with three different pathogens: *R. solanaearum* (Figure 13 A and 13B), *H. arabidopsidis* (Figure 13 C) and *M. incognita* (Figure 13D).

- *Analysis of R. solanaearum proliferation (Figure 13 A and 13B)*

Four week-old plants were root-inoculated with a solution containing 10^7 bacteria per ml of the virulent bacterial isolate RD15. For analyzing bacterial internal growth *R. solanaearum*, the procedure described above was applied (see the legend to Figure 7C in connection with Example 7). *R. solanacearum* was re-extracted at different time points after inoculation to determine pathogen titers. For each time point, triplicate assays were performed for each *A. thaliana* line.

20 - *Analysis of H. arabidopsidis proliferation (Figure 13 C)*

Plants were spray-inoculated with 40,000 spores/ml and cotyledons were collected 5 days post inoculation. Intercellular growth and branching of *H. arabidopsidis* was microscopically analyzed by trypan blue-staining. Infected seedlings were covered with trypan blue solution (0,01 % w/v in 10 % phenol, 10% lactic acid, 10 % water, 20 % glycerol, and 50 % ethanol, v/v), boiled for 3 min, stored at room temperature overnight, and bleached with chloral hydrate at 2,5 g/ml, before being mounted in 50 % glycerol onto microscope slides, and photographed.

- *Analysis of M. incognita proliferation (Figure 13 D)*

30 For morphological analyses, nematode-infected roots of *pskr1-2*, *PSKR1-OE* and wild-type plants (ecotype Ws) were fixed in 2% glutaraldehyde in 50mM Pipes buffer (pH 6.9) on 7, 14 and 21 days post inoculation and then dehydrated and embedded in

Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany) as described by the manufacturer. Embedded tissues were sectioned (3µm) and stained in 0.05% toluidine blue, mounted in Depex (Sigma) and microscopy was performed using bright field optics. Images were collected with a digital camera (AxioCam; Zeiss). Tissue sections
 5 through galls on 7 days post inoculation from *pskr1-2* and *PSKR1-OE* showed no difference in gall and giant cells formation in comparison with control. At later stages of gall development (14 and 21 days post inoculation) the giant cells from *pskr1-2* mutant plants were significantly smaller. For giant cell surface measurements, serial sections stained with toluidine blue were examined using the AxioVision V 4.8.1.0 software.
 10 Finally, giant cell development upon *M. incognita* infection was quantified on numerized micrographs taken from thin-sectioned, toluidine blue-stained roots isolated from the different lines. The three biggest giant cells per gall from at least 50 galls per phenotype were chosen for measurements. Galls from *pskr1-2* mutant plants contain significantly smaller giant cells in comparison to control plants at 14 days post
 15 inoculation.

Results:

Figure 13 clearly shows that *PSKR1* suppression causes reduced proliferation of the following pathogens: *R. solanacearum* (bacterium), *H. arabidopsidis* (oomycete), and
 20 *M. incognita* (nematode).

In particular, Figures 13A and 13B show that multiplication of the bacterium *R. solanacearum* is strongly reduced in the absence of *PSKR1* in the *pskr1-2* mutant. Bacterial multiplication is restored to the wild-type level upon introduction of a functional *PSKR1* gene into the *pskr1-2* genetic background (complemented line
 25 *Cppskr1-2*), and increased in a line overexpressing *PSKR1* under the control of the constitutive 35S promoter (overexpressing line *PSKR1-OE*). In conclusion, bacterial multiplication is drastically reduced (~1,000- fold) in the *pskr1-2* mutant, restored in the complemented line, and increased (~2-fold) in the overexpressing line.

Figure 13C shows that the network and hyphal branching of the oomycete *H. arabidopsidis* is strongly reduced in the absence of *PSKR1* in the *pskr1-2* mutant, but
 30 becomes aberrant upon overexpression of *PSKR1* in the *PSKR1-OE* line.

Figure 13D shows that the reduced egg mass production by the nematode *M. incognita* is a consequence of reduced giant cell sizes in the absence of PSKR1.

Example 11: Generation of mutant *Solanum lycopersicum* lines for the PSKR1 gene by the TILLING strategy

The tomato SlPSKR sequence (SEQ ID N0: 67) was used as the target for a TILLING strategy to obtain tomato lines with an inactive PSKR1 protein with reduced susceptibility to plant pathogens.

The TILLING method is known *per se* in the art, including the preparation of genomic DNA, the generation of DNA pools and superpools, the targeted identification of single nucleotide exchanges, and the deconvolution steps to obtain individuals (see e.g., Piron *et al.*, 2010).

The inventors have tested plants from the parental M82 tomato line for interaction phenotypes with the oomycete, *Phytophthora parasitica*, and the root-knot nematode, *Meloidogyne incognita*. The parental line was fully susceptible to both pathogens. *SlPSKR1* was selected as target gene for the TILLING approach, because it does not contain introns. The inventors have defined two genomic regions of *SlPSKR1* to be targeted as shown in Figure 14. The first target corresponds to the sequence coding for the extracellular LRR domain of the protein. The second target corresponds to the sequence coding for the C-terminal region of the protein, including membrane-spanning and kinase domains. Target 1 and 2 amplicons were generated with 2 sets of primers each, one set being specific for the target, and a second nested on the first and allowing to generate adaptors. Universal M13 primers that were labelled at the 5'end with the infra-red dyes IRD700 and IRD800 were used to generate the final amplicons that were analyzed for heteroduplexes after digestion by Endo1. Primers used for *SlPSKR1* TILLING having sequences of SEQ ID NO: 76 to 85, are shown in the Table below:

Target	Primer Name	Sequence 5' > 3'	Characteristics
1, LRR domain	SIPSKR1-F3	GGGTGTGTTGCAAGTTTGTGTGATC	Target-specific, PCR 1
1, LRR domain	SIPSKR1-R3	CAAGTCTAACAGTTGCAGTTTGAGC	Target-specific, PCR 1
1, LRR domain	SIPSKR1-M13-F4	CACGACGTTGTAACGACTTACAAGCACAATCTC	Generates adaptor, PCR 2
1, LRR domain	SIPSKR1-M13-R2	GGATAACAATTTACACAGGCTGAGGAACAACCTCC	Generates adaptor, PCR 2
2, TM-kinase domain	SIPSKR1-F4-2	GAGGGCAACCAAGGACTCTGCGGTG	Target-specific, PCR 1
2, TM-kinase domain	SIPSKR1-R6	GCAGGACATCCGCTGGAATATAAG	Target-specific, PCR 1
2, TM-kinase domain	SIPSKR1-M13-F5	CACGACGTTGTAACGACCTGTCGAAATGCCAGC	Generates adaptor, PCR 2
2, TM-kinase domain	SIPSKR1-M13-R5	GGATAACAATTTACACAGGCTGAGGAACAACCTCC	Generates adaptor, PCR 2
Adaptor	M13F700	CACGACGTTGTAACGAC	IRD700-labeled universal
Adaptor	M13R800	GGATAACAATTTACACAGG	IRD800-labeled universal

The screen of 7 x 96-well titer plates containing genomic DNA from 8 individuals/well revealed 23 potential mutations in target 1 plus 14 potential mutations in target 2 (= 37 potential mutants). The deconvolution procedure led to the identification of the first 6 individual lines, 4 harboring single nucleotide changes within target 1, and 2 with single nucleotide changes within target 2.

Seeds from the 6 individual lines were sown, to generate homozygous plants with reduced susceptibility to plant pathogens. Domains, targets, primer attachment sites, and the sites of the obtained 6 mutations within SIPSKR1 are indicated in Figure 14.

Conclusions

Altogether, the expression data and the phenotypical data indicate that *PSK* and *PSKR* genes are negative regulators of resistance to plant pathogens and that the enhanced resistance to pathogens and the reduced susceptibility to infection, which is observed in the *PSK* and *PSKR* knock-out mutants is due to a “loss of function” mutation in the *PSK* or *PSKR* gene.

Enhanced resistance of *pskr1* mutants is not a consequence of constitutively activated, or pathogen-triggered defense responses and the mutants thus present a loss-of-susceptibility phenotype, rather than a gain of resistance. As shown in Figure 12, the activation of salicylic acid (SA)-, jasmonic acid (JA)-, and ethylene (JA/ethylene)-mediated defense signaling pathways in *Arabidopsis* are independent of *PSKR1*. Marker

genes for SA-, JA, and JA/ethylene-mediated signaling pathways were *PR1a* (At2g14610) *PDF1.2* (At5g44420), and *PR4* (At3g04720), respectively. Expression of these defense-related genes was analyzed by quantitative real-time RT-PCR in wild type (Ws), mutant (*pskr1-2*), and transgenic PSKR1 overexpressor (PSKR1-OE) plants upon
 5 spray treatment of cotyledons with water, or with conidiospore suspensions (40,000 spores/ml) of the *H. arabidopsidis* isolate Emwa1.

Moreover, data obtained with *pskr1* mutants confirm a correlation between PSKR1 suppression and reduced pathogen proliferation.

10

Furthermore, data obtained with plants overexpressing *PSK* or *PSKR* confirm a correlation between expression of PSK and susceptibility to infection since overexpression of *PSK* or *PSKR1* increases susceptibility to pathogen infection.

15 This is the first example ever found of a plant growth factor negatively regulating disease resistance.

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CLAIMS

1. A method for protecting a plant against pathogens, comprising a step of inhibiting permanently or transiently phytosulfokine (PSK) function in said plant or an ancestor thereof.
2. A method for increasing pathogen resistance in a plant, comprising a step of inhibiting permanently or transiently phytosulfokine (PSK) function in said plant or an ancestor thereof.
3. A method for decreasing pathogen proliferation in a plant, comprising a step of inhibiting permanently or transiently phytosulfokine (PSK) function in said plant or an ancestor thereof.
4. The method of any one of claims 1 to 3, wherein said plant has a defective PSK gene, a defective PSK peptide, a defective PSK receptor (PSKR) gene and/or a defective PSKR receptor.
5. The method of claim 4, wherein said PSK or PSKR gene is defective as a result of a deletion, insertion and/or substitution of one or more nucleotides, site-specific mutagenesis, ethyl methanesulfonate (EMS) mutagenesis, targeting induced local lesions in genomes (TILLING), EcoTILLING, knock-out techniques, inactivation with a ribozyme or antisense nucleic acid, or by gene silencing induced by RNA interference.
6. The method of claim 5, wherein the PSK and/or PSKR gene(s) is/are fully or partially deleted.
7. The method of any one of claims 4 to 6, wherein each copy of the PSK gene, when present in several copies in said plant cells, is rendered defective.
8. The plant of any one of claims 4 to 6, wherein the PSKR1 and PSKR2 genes are rendered defective in said plant cells.

9. The method of claim 4, wherein said PSK peptide is inactivated by exposing said plant to, or by expressing into said plant, an antibody directed against the PSK peptide, or a soluble PSKR receptor.

5

10. The method of any one of the preceding claims, wherein said plant pathogen is selected from fungi, oomycetes, nematodes or bacteria.

11. The method of any one of the preceding claims, wherein said plant is a dicot, preferably selected from *Solanaceae* (e.g. tomato), *Liliaceae* (e.g. asparagus), *Apiaceae* (e.g. carrot), *Chenopodiaceae* (e.g. beet), *Vitaceae* (e.g. grape), *Fabaceae* (e.g. soybean), *Cucurbitaceae* (e.g. Cucumber) or *Brassicaceae* (e.g. rapeseed, *Arabidopsis thaliana*), or a monocot, preferably selected from the cereal family *Poaceae* (e.g. wheat, rice, barley, oat, rye, sorghum or maize).

15

12. A method for producing a plant having increased resistance to plant pathogens, wherein the method comprises the following steps:

- (a) inactivation of PSK and/or PSKR gene(s) in plant cells;
- (b) optionally, selection of plant cells of step (a) with defective PSK and/or PSKR gene(s);
- (c) regeneration of plants from cells of step (a) or (b); and
- (d) optionally, selection of a plant of (c) with increased resistance to pathogens, said plant having defective PSK or PSKR gene(s).

20

13. The method according to claim 12, wherein, in step (a), said PSK or PSKR gene is inactivated by deletion, insertion and/or substitution of one or more nucleotides, site-specific mutagenesis, ethyl methanesulfonate (EMS) mutagenesis, targeting induced local lesions in genomes (TILLING), EcoTILLING, knock-out techniques, or by gene silencing induced by RNA interference.

30

14. The method according to claim 12 or 13, wherein the plant is a dicot, preferably selected from the families *Solanaceae* (e.g. tomato), *Liliaceae* (e.g. asparagus),

Apiaceae (e.g. carrot), *Chenopodiaceae* (e.g. beet), *Vitaceae* (e.g. grape), *Fabaceae* (e.g. soybean), *Cucurbitaceae* (e.g. Cucumber) or *Brassicaceae* (e.g. rapeseed, *Arabidopsis thaliana*), or a monocot, preferably selected from the cereal family *Poaceae* (e.g. wheat, rice, barley, oat, rye, sorghum or maize).

5

15. The method according to any one of claims 12 to 14, wherein said plant pathogens are selected from fungi, oomycetes, nematodes or bacteria.

16. Use of an RNAi molecule that inhibits the expression of a *PSK* or *PSKR* gene for increasing resistance of plants or plant cells to plant pathogens and/or for decreasing plant pathogen proliferation in plants or plant cells and/or for protecting plants or plant cells against plant pathogens.

17. A method of identifying a molecule that modulates the *PSKR* gene expression, the method comprising:

- (a) providing a cell comprising a nucleic acid construct that comprises a *PSKR* gene promoter sequence operably linked to a reporter gene;
- (b) contacting the cell with a candidate molecule;
- (c) measuring the activity of *PSKR* promoter by monitoring of the expression of a marker protein encoded by the reporter gene in the cell;
- (d) selecting a molecule that modulates the expression of the marker protein.

18. The method of claim 17, wherein the molecule inhibits the expression of *PSKR*.

19. Use of a molecule selected according to claim 17 or 18 for increasing resistance of plants to plant pathogens and/or for decreasing plant pathogen proliferation in plants or plant cells and/or for protecting plants or plant cells against plant pathogens.

20. An antibody that specifically binds a *PSK* peptide or receptor, or a fragment or derivative of such an antibody having essentially the same antigenic specificity.

ABSTRACT

The present invention relates to plant genes involved in negative regulation of resistance to plant pathogens and uses thereof. More particularly, the invention relates to plants
5 having a defective phytosulfokine (PSK) function and exhibiting an increased resistance to plant pathogens. The invention also relates to methods for producing modified plants resistant to various diseases. Furthermore, the invention relates to plants having a defective PSK receptor (PSKR) function, and to methods of screening and identifying molecules that modulate PSKR expression or activity.

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 <210> 3
 <211> 87

<212> PRT
<213> Arabidopsis thaliana

<400> 3

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1 5 10 15

Leu Leu Leu Ser Met Ala Ser Ser Val Ile Leu Arg Glu Asp Gly Phe
20 25 30

Ala Pro Pro Lys Pro Ser Pro Thr Thr His Glu Lys Ala Ser Thr Lys
35 40 45

Gly Asp Arg Asp Gly Val Glu Cys Lys Asn Ser Asp Ser Glu Glu Glu
50 55 60

Cys Leu Val Lys Lys Thr Val Ala Ala His Thr Asp Tyr Ile Tyr Thr
65 70 75 80

Gln Asp Leu Asn Leu Ser Pro
85

<210> 4
<211> 87
<212> PRT
<213> Arabidopsis thaliana

<400> 4

Met Ala Asn Val Ser Ala Leu Leu Thr Ile Ala Leu Leu Leu Cys Ser
1 5 10 15

Thr Leu Met Cys Thr Ala Arg Pro Glu Pro Ala Ile Ser Ile Ser Ile
20 25 30

Thr Thr Ala Ala Asp Pro Cys Asn Met Glu Lys Lys Ile Glu Gly Lys
35 40 45

Leu Asp Asp Met His Met Val Asp Glu Asn Cys Gly Ala Asp Asp Glu
50 55 60

Asp Cys Leu Met Arg Arg Thr Leu Val Ala His Thr Asp Tyr Ile Tyr
65 70 75 80

Thr Gln Lys Lys Lys His Pro
85

<210> 5
<211> 81
<212> PRT
<213> Arabidopsis thaliana

<400> 5

Met Lys Gln Ser Leu Cys Leu Ala Val Leu Phe Leu Ile Leu Ser Thr
1 5 10 15

Ser Ser Ser Ala Ile Arg Arg Gly Lys Glu Asp Gln Glu Ile Asn Pro
20 25 30

Leu Val Ser Ala Thr Ser Val Glu Glu Asp Ser Val Asn Lys Leu Met
35 40 45

Gly Met Glu Tyr Cys Gly Glu Gly Asp Glu Glu Cys Leu Arg Arg Arg
50 55 60

Met Met Thr Glu Ser His Leu Asp Tyr Ile Tyr Thr Gln His His Lys
65 70 75 80

His

<210> 6
<211> 79
<212> PRT
<213> Arabidopsis thaliana

<400> 6

Met Gly Lys Phe Thr Thr Ile Phe Ile Met Ala Leu Leu Leu Cys Ser
1 5 10 15

Thr Leu Thr Tyr Ala Ala Arg Leu Thr Pro Thr Thr Thr Thr Ala Leu
20 25 30

Ser Arg Glu Asn Ser Val Lys Glu Ile Glu Gly Asp Lys Val Glu Glu
35 40 45

Glu Ser Cys Asn Gly Ile Gly Glu Glu Glu Cys Leu Ile Arg Arg Ser
50 55 60

Leu Val Leu His Thr Asp Tyr Ile Tyr Thr Gln Asn His Lys Pro
65 70 75

<210> 7
<211> 77
<212> PRT
<213> Arabidopsis thaliana

<400> 7

Met Val Lys Phe Thr Thr Phe Leu Cys Ile Ile Ala Leu Leu Leu Cys
1 5 10 15

Ser Thr Leu Thr His Ala Ser Ala Arg Leu Asn Pro Thr Ser Val Tyr
20 25 30

Pro Glu Glu Asn Ser Phe Lys Lys Leu Glu Gln Gly Glu Val Ile Cys
35 40 45

Glu Gly Val Gly Glu Glu Glu Cys Phe Leu Ile Arg Arg Thr Leu Val
50 55 60

Ala His Thr Asp Tyr Ile Tyr Thr Gln Asn His Asn Pro
65 70 75

<210> 8
<211> 84
<212> PRT
<213> Populus trichocarpa

<400> 8

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

Leu Cys Ser Thr Leu Thr Tyr Ala Ala Arg Pro Glu Pro Gly Phe Pro
20 25 30

Asn Gly Ser Leu Ala Lys Asn Gln Gln Lys Val Val Asp Ala Glu His
35 40 45

Ala Glu Val Met Glu Glu Ser Cys Glu Gly Val Gly Glu Glu Glu Cys
50 55 60

Leu Met Arg Arg Thr Leu Ala Ala His Thr Asp Tyr Ile Tyr Thr Gln
65 70 75 80

Lys His Lys Pro

<210> 9
<211> 90
<212> PRT
<213> Populus trichocarpa

<400> 9

Met Lys Leu Ser Leu Asn Tyr Lys Ala Leu Leu Leu Ile Leu Leu Val
1 5 10 15

Leu Val Tyr Ser Ser Lys Leu Ser Ala Arg Phe Leu Leu Ser Lys Gln
20 25 30

Gly Gln Glu Glu Val Asn Val Asp Gly Ile Thr Ser Glu Gly Thr Glu
35 40 45

Asp Ser Glu Leu Met Asn Gln Leu Thr Gly Leu Glu Leu Cys Asp Gly
50 55 60

Gly Asp Glu Glu Cys Leu Thr Arg Arg Ile Ile Ala Glu Ala His Leu
65 70 75 80

Asp Tyr Ile Tyr Thr Gln Asn His Lys Pro
85 90

<210> 10
<211> 71
<212> PRT
<213> Populus trichocarpa

<400> 10

Ile Leu Leu Phe Ser Phe Thr Leu Thr Ser Ala Ala Arg Pro Glu Pro
1 5 10 15

Ala Phe Ala Asp Val Thr Pro Met Glu Thr Leu Tyr Gly Asp Asn Ala
20 25 30

Glu Ala Glu Thr Val Glu Met Glu Lys Ser Cys Glu Gly Val Gly Glu
35 40 45

Asp Glu Cys Leu Thr Arg Arg Thr Leu Ala Ala Gln Ile Asp Tyr Ile
50 55 60

Tyr Thr Gln Lys His Lys Pro
65 70

<210> 11
<211> 85
<212> PRT
<213> Populus trichocarpa

<400> 11

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

Leu Cys Ser Thr Ile Thr Tyr Ala Ala Arg Pro Glu Pro Gly Phe Pro
20 25 30

Gly Gly Ser Leu Ala Lys Asn Gln His Lys Val Val Glu Ala Glu His
35 40 45

Ala Glu Val Met Glu Glu Ile Ser Cys Glu Gly Leu Gly Glu Glu Glu
50 55 60

Cys Leu Met Arg Arg Thr Leu Ala Ala His Thr Asp Tyr Ile Tyr Thr
65 70 75 80

Gln Lys Asn Asn Pro
85

<210> 12
<211> 76
<212> PRT
<213> Populus trichocarpa

<400> 12

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

Thr Leu Thr Tyr Ala Ala Arg Pro Arg Pro Val Pro Val Leu Ser Asp
20 25 30

Glu Pro Leu Asp Val Lys Ala Asp Glu Ala Ala Val Val Glu Ser Cys
35 40 45

Glu Gly Leu Gly Val Glu Ala Cys Leu Ala Arg Arg Thr Leu Ala Ala
50 55 60

Gln Val Asp Tyr Ile Tyr Thr Gln Lys Gln Asn Pro
65 70 75

<210> 13
<211> 95
<212> PRT
<213> Populus trichocarpa

<400> 13

Met Lys Gln Thr Leu His Tyr Lys Ala Leu Leu Leu Phe Leu Leu Val
1 5 10 15

Leu Val His Ser Ser Lys Leu Ser Ala Arg Phe Leu Leu Ser Lys Gln
20 25 30

Gly Lys Glu Asp Leu Asn Leu Lys Glu Ile Thr Ser Glu Gly Thr Phe
35 40 45

Ala Gln Thr Glu Asp Ser Glu Leu Ile Thr Asn Gln Leu Met Gly Leu
50 55 60

Glu Val Cys Arg Gly Gly Asp Glu Glu Cys Phe Lys Arg Arg Ile Ile
65 70 75 80

Ala Glu Ala His Leu Asp Tyr Ile Tyr Thr Gln His His Lys Pro
85 90 95

<210> 14
<211> 101
<212> PRT
<213> Oryza sativa

<400> 14

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

Leu Cys Leu Ile Cys Thr Arg Ser Gln Ala Ala Arg Pro Glu Pro Gly
20 25 30

Ser Ser Gly His Lys Ser Gln Gly Val Val Ala Ser Ser Ile Ala His
35 40 45

Gln Lys Ser Val Gly Ser Ser Gly Ile Gly Val Glu Met His Gln Gly
50 55 60

Glu Pro Asp Gln Ala Val Glu Cys Lys Gly Gly Glu Ala Glu Glu Glu
65 70 75 80

Cys Leu Met Arg Arg Thr Leu Val Ala His Thr Asp Tyr Ile Tyr Thr
85 90 95

Gln Gly Asn His Asn
100

<210> 15
<211> 53
<212> PRT
<213> Oryza sativa

<400> 15

Met Val Val Glu Leu Gly Gln Leu Asn Pro Ala Lys Leu Pro Val Val
1 5 10 15

Glu Arg Gly Asn Tyr Asp Gly Arg Val Glu Gly Cys Glu Glu Asp Asp
20 25 30

Cys Leu Val Glu Arg Leu Leu Val Ala His Leu Asp Tyr Ile Tyr Thr
35 40 45

Gln Gly Lys His Asn
50

<210> 16
<211> 83
<212> PRT
<213> Oryza sativa

<400> 16

Met Ala Ala Arg Thr Val Ala Val Ala Ala Ala Leu Ala Val Leu Leu
1 5 10 15

Ile Phe Ala Ala Ser Ser Ala Thr Val Ala Met Ala Gly Arg Pro Thr
20 25 30

Pro Thr Thr Ser Leu Asp Glu Glu Ala Ala Gln Ala Ala Ala Gln Ser
35 40 45

Glu Ile Gly Gly Gly Cys Lys Glu Gly Glu Gly Glu Glu Glu Cys Leu
50 55 60

Ala Arg Arg Thr Leu Thr Ala His Thr Asp Tyr Ile Tyr Thr Gln Gln
65 70 75 80

His His Asn

<210> 17
<211> 119
<212> PRT
<213> Oryza sativa

<400> 17

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

Ala Leu Leu Leu Leu Phe Ala Leu Cys Phe Phe Ser Phe His Phe Ala
20 25 30

Ala Ala Ala Arg Ala Val Pro Arg Asp Glu His Gln Glu Asn Gly Gly
35 40 45

Val Lys Ala Val Ala Ala Val Ala Ala Asp Gln Leu Val Leu Gln Leu
50 55 60

Glu Gly Asp Thr Gly Asn Gly Asp Glu Val Ser Glu Leu Met Gly Ala
65 70 75 80

Ala Glu Glu Glu Ala Ala Ala Cys Glu Glu Gly Lys Asn Asn Asp Glu
85 90 95

Cys Val Gln Arg Arg Leu Leu Ser Asp Ala His Leu Asp Tyr Ile Tyr
100 105 110

Thr Gln His Lys Asn Lys Pro
115

<210> 18
<211> 94
<212> PRT
<213> Oryza sativa

<400> 18

Met Ala Pro Pro Arg Cys Thr Ala Leu Leu Leu Leu Ala Ser Leu Leu
1 5 10 15

Leu Phe Phe Leu Cys Ile Ser Ala Thr His Glu Ala Ala Arg Thr Ala
20 25 30

Ser Gly Gln Pro Ile Gln Glu Gln Glu Gln Glu Gln His Gly Lys Val
35 40 45

Glu Glu Glu Thr Met Ala Ala Ser Phe Ala Ala Val Glu Glu Gln Cys
50 55 60

Gly Gly Glu Glu Gly Glu Glu Glu Glu Cys Leu Met Arg Arg Thr Leu
65 70 75 80

Val Ala His Thr Asp Tyr Ile Tyr Thr Gln Gly Asn His Asn
85 90

<210> 19
<211> 102
<212> PRT
<213> Oryza sativa

<400> 19

Met Arg Pro Thr Gly Arg Arg Ser Ser Pro Pro Val Ala Ala Ala Leu
1 5 10 15

Ala Leu Leu Leu Leu Val Leu Phe Phe Phe Ser His Cys Ala Ser
20 25 30

Ala Ala Arg Pro Leu Pro Ala Ser Ala Ala Ala Glu Leu Val Leu Gln
35 40 45

Asp Gly Ala Thr Gly Asn Gly Asp Glu Val Ser Glu Leu Met Gly Ala
50 55 60

Ala Glu Glu Glu Ala Ala Gly Leu Cys Glu Glu Gly Asn Glu Glu Cys
65 70 75 80

Val Glu Arg Arg Met Leu Arg Asp Ala His Leu Asp Tyr Ile Tyr Thr
85 90 95

Gln Lys Arg Asn Arg Pro
100

<210> 20
<211> 83
<212> PRT
<213> Vitis vinifera

<400> 20

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

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

Val Thr Pro Met Lys Ile Gln His Gly Asp Val Asp Glu Ala Lys Thr
35 40 45

Val Glu Val Glu Glu Ser Cys Glu Gly Val Gly Glu Glu Glu Cys Leu
50 55 60

Met Arg Arg Thr Leu Ala Ala His Thr Asp Tyr Ile Tyr Thr Gln Lys
65 70 75 80

Lys Asn Pro

<210> 21

<211> 96

<212> PRT

<213> Vitis vinifera

<400> 21

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

Leu Ile Phe Ser Ser Ser Ser Lys Ser Ser Ala Arg Leu Leu Ile Thr
20 25 30

Lys Gln Gly Glu Glu Gly Val Lys Leu Lys Glu Leu Ile Asn Gly Val
35 40 45

Ser Leu Leu Glu Met Glu Gly Asn Asp Ser Phe Glu Gln Leu Met Gly
50 55 60

Val Glu Asp Cys Glu Asn Gly Asp Glu Glu Cys Leu Lys Arg Arg Ile
65 70 75 80

Ile Ser Glu Ala His Leu Asp Tyr Ile Tyr Thr Gln His His Lys Pro
85 90 95

<210> 22

<211> 90
<212> PRT
<213> Vitis vinifera

<400> 22

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

Leu Leu Tyr Ser Tyr Ser Gly Ser Ala Arg Leu Leu Ala Thr Lys Gln
20 25 30

Asp Glu Gln Val Val Met Gly Glu Trp Met Thr His Ala Gly Thr Ser
35 40 45

Lys Gly Glu Asp Val Leu Asn Leu Met Gly Leu Glu Lys Cys His Glu
50 55 60

Asn Asp Glu Glu Cys Leu Lys Arg Arg Met Val Ala Glu Ala His Leu
65 70 75 80

Asp Tyr Ile Tyr Thr Gln His His Lys Pro
85 90

<210> 23
<211> 83
<212> PRT
<213> Vitis vinifera

<400> 23

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

Thr Leu Ser Ser Ala Ala Arg His Glu Pro Thr Phe Val Thr Asp Ser
20 25 30

Ala Val Lys Phe Gln Tyr Glu Glu Val Glu Ala Glu Lys Ser Met Glu
35 40 45

Val Val Gly Gly Ser Cys Glu Gly Ala Ala Asp Lys Asp Glu Cys Leu
50 55 60

Met Arg Arg Thr Leu Ala Ala His Thr Asp Tyr Ile Tyr Thr Gln Lys
65 70 75 80

Gln Lys Pro

<210> 24
 <211> 82
 <212> PRT
 <213> Vitis vinifera

<400> 24

Met Ser Pro Lys Val Ala Thr Phe Phe Ile Leu Ala Leu Phe Leu Cys
 1 5 10 15

Ser Thr Leu Thr Tyr Ala Ala Arg Pro Gln Pro Ala Ser Pro His Asp
 20 25 30

Phe Pro Gly Lys Thr Gln His Gly Gly Val Glu Ala Glu Arg Ala Glu
 35 40 45

Val Val Asp Gly Asn Cys Glu Gly Val Gly Glu Asp Glu Cys Leu Met
 50 55 60

Arg Arg Thr Leu Ala Ala His Ile Asp Tyr Ile Tyr Thr Gln Lys Glu
 65 70 75 80

Lys Pro

<210> 25
 <211> 792
 <212> DNA
 <213> Arabidopsis thaliana

<400> 25

agaagaagaa gaagaagaaa aataaaagaa atgatgaaga cgaaaagtga agtgttgatc	60
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ggttttgctc ctctaaacc atctcccacc acacatgtaa gttcgtcaat atatgtgcat	180
cacatatagc ggaattattt ttcgataaca tgaatacttg ttgattactg tgcatacataa	240
tagaagttat gcgataacgt tttgaaagag tgaaaacatg aataagtggg atgcgatcca	300
tcaccattat agctatgtat gtttgataac gatattttgga taagaatggg tataagttgt	360
aatattgggt tcaacatatg gtggattggg gattataaaa aaattgaata cagaaatttt	420
attgaaagat ataaatgaat aattttttta caaaaaata tatatataaa tgaataaatt	480
atagtgattc atcatctcac tacttttttt ttcttggtgg atctaggaga aagcaagtac	540

taaaggtgac agagatggag tagagtgcaa gaattcagac agtgaagaag aatgtcttgt	600
gaagaaaaca gtagctgctc acaccgatta catctataca caagatttaa acctatctcc	660
ttgaaacaag aactattact ctttgtcttt tattttagcaa actatatgca taagcctttg	720
aaaggagctt ctgcacctca aactcagctc ttcatgttct ttgttctttt tttcataagc	780
ttcgtttaat gt	792

<210> 26
 <211> 692
 <212> DNA
 <213> Arabidopsis thaliana

<400> 26	
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ctgcccggcc cgaaccggcc atctccatct ctatcacgac tgctgccgat ccatgtaaca	180
tggttagtct gattcacatg ccatgcatga atcaatttcc atatataaac acaaaataac	240
caatatcaga gcttctaaat ttttaaaaat atttaacaca tcgaataggt ttaataaatt	300
ttttgtaatg tatgatgatt actttggcag gagaagaaga tagaaggaaa attagatgac	360
atgcatatgg tagacgaaaa ctgtggtgca gacgacgaag attgcttaat gaggaggact	420
ttggtcgctc atactgatta catctatacc cagaagaaga agcatccttg attttcactt	480
actcatttct aacaaacttt tgctcaagat tatgtaattt atgtcatcct taattagtat	540
ttgtcacata caagatcaag atagttatga ttgtgattat ttatatgtat tttggatgat	600
atattgtaga actagaagtt tgacaataaa aagtttatag tgtcgttgta tgtttaagaa	660
gtttgacaat aaaaacttta tagtgttggt gc	692

<210> 27
 <211> 959
 <212> DNA
 <213> Arabidopsis thaliana

<400> 27	
actttttttc tctctctctc tctcaaccaa gaacatgaag caaagcttgt gcctggcagt	60
tctcttcctc attttatcaa caagttcatc tgcaattcga agaggatatgt tgttgaatac	120
agtaatgatt agtttcttaa aattaatatt atcaataacc aaaatcatct tccttcaact	180
ctaaatcttt atatgtaatc aggaaaagaa gatcaagaga taaatccatt agtttcagct	240
acatcagtgg aagaggactc agttaatgta agttcatcta aatttttccc taagataaat	300

aaacaatttg	cttattttat	tttcctaaaa	tatgctatca	gatgcatacc	aattaacatt	360
ttcaaagtta	atttcttaat	gatatttcag	agtaatgttc	tacttttcta	aattgaaatt	420
tgaacttgaa	agaattcaac	tcacttttaa	taattacaaa	aaaaagatat	gagaaactca	480
actcaatggg	gtatttttat	ttcttcctaa	ttgtagtaat	aagtatctca	taattgaata	540
gggttaggga	gttgacaaaa	aaaaaaaagg	gttaaggaaa	ctcaaagtag	tttagtaa	600
ttatatgcag	aatcagagat	caatatttta	acctttttgt	ctttgtaaaa	acagaaattg	660
atggggatgg	aatattgtgg	agaaggagat	gaagaatggt	tgaggagaag	gatgatgacg	720
gaatctcact	tagactatat	ttacacacag	caccataagc	attgacatta	attgttatca	780
ttttgattaa	tttgcataca	tatatgtatg	tgtatgtttt	gccccaaaa	aaaaagtgt	840
tgttttgtgt	tgatgttact	tagatatatt	tgtattgtat	atgagtccta	tgtgacaaaa	900
cagctccaag	aaaccaatat	ttttgttttt	aattagagaa	aaaatggtga	ttgaataaa	959

<210> 28
 <211> 698
 <212> DNA
 <213> Arabidopsis thaliana

<400> 28	
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	ttt 60
tgcaaatcag	tatgggtaag ttcacaacca ttttcatcat ggctctcctt ctttgctcta
	ttt 120
cgctaacct	cgcagcaagg ctgactccga cgacaaccac cgctttgtcc agagaaaact
	ttt 180
ccgtcaagg	tgcgttaactt ctttgtcttt ttcagtatag tactagtcga aacatatctg
	ttt 240
caattgcaa	acaaagaatt aatctatcgc agtatatgtc aaagtttcta tatatagtac
	ttt 300
aaaacaaaa	acaaaaaga gtttgcattg atgctcctta agatttggtt cgtgtaatag
	ttt 360
attatataat	atcacacgat ttgtttat
	ttt 420
acgttcata	gttggtgtat atattatgta taggaaattg aaggagacaa ggttgaagaa
	ttt 480
gaaagctgc	acggaattgg agaagaagaa tgtttgataa gacgaagcct tgttcttcac
	ttt 540
accgattaca	tttatactca gaatcacaag ccctaagttt ctgtattaga gcaattaatt
	ttt 600
aactaattac	attatcaaac ctatcactgt agtactttct gttttctggt cgtctttttg
	ttt 660
ttttgtttt	tttatgttat ggctatttta aagtttca
	ttt 698

<210> 29
 <211> 762
 <212> DNA
 <213> Arabidopsis thaliana

<400> 29
tcattctttat tgataatttct caatataatt gatctctgtc tcatagttat aaaaagaatt 60
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tcttcttctc tgctccacgc taacacacgc atcagctcgg ctcaatccaa catccgttta 180
tccagaagaa aactccttca aggtattaac ctcatgctca tgggtgtatat catcagtata 240
tgtgtacata ggaaacatga tcgaaaaggc tttaatcggt taaataacaa ggatgtacaa 300
ttttcctgaa attaaagact agtaaataata tatgggtttca gctaaagatt gtctgattac 360
cataaaagaa aaagaatcat tatcgagatt aacaattgag tcacgtgcgt ataactctttt 420
cttatgcgca gaaactagaa cagggagagg taatctgtga aggtggttga gaagaagaat 480
gcttcttgat acgaagaact ttagttgctc aactgatta catctacact caaaaccaca 540
atccctaaat gatcaattag cttcttatta ttggttgata cttggattag taatcagtat 600
atatatccac atatatatat gatgtttttc ttatatccat atatttagct tcttaatat 660
gttgcacttt gtactttctt aattattaac caaaagtact acacatttat atacttatca 720
cttaatctga atatacatat gcgtataaga ttattcaatt at 762

<210> 30
<211> 1008
<212> PRT
<213> Arabidopsis thaliana

<400> 30

Met Arg Val His Arg Phe Cys Val Ile Val Ile Phe Leu Thr Glu Leu
1 5 10 15

Leu Cys Phe Phe Tyr Ser Ser Glu Ser Gln Thr Thr Ser Arg Cys His
20 25 30

Pro His Asp Leu Glu Ala Leu Arg Asp Phe Ile Ala His Leu Glu Pro
35 40 45

Lys Pro Asp Gly Trp Ile Asn Ser Ser Ser Ser Thr Asp Cys Cys Asn
50 55 60

Trp Thr Gly Ile Thr Cys Asn Ser Asn Asn Thr Gly Arg Val Ile Arg
65 70 75 80

Leu Glu Leu Gly Asn Lys Lys Leu Ser Gly Lys Leu Ser Glu Ser Leu
85 90 95

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

Lys Asp Ser Ile Pro Leu Ser Ile Phe Asn Leu Lys Asn Leu Gln Thr
115 120 125

Leu Asp Leu Ser Ser Asn Asp Leu Ser Gly Gly Ile Pro Thr Ser Ile
130 135 140

Asn Leu Pro Ala Leu Gln Ser Phe Asp Leu Ser Ser Asn Lys Phe Asn
145 150 155 160

Gly Ser Leu Pro Ser His Ile Cys His Asn Ser Thr Gln Ile Arg Val
165 170 175

Val Lys Leu Ala Val Asn Tyr Phe Ala Gly Asn Phe Thr Ser Gly Phe
180 185 190

Gly Lys Cys Val Leu Leu Glu His Leu Cys Leu Gly Met Asn Asp Leu
195 200 205

Thr Gly Asn Ile Pro Glu Asp Leu Phe His Leu Lys Arg Leu Asn Leu
210 215 220

Leu Gly Ile Gln Glu Asn Arg Leu Ser Gly Ser Leu Ser Arg Glu Ile
225 230 235 240

Arg Asn Leu Ser Ser Leu Val Arg Leu Asp Val Ser Trp Asn Leu Phe
245 250 255

Ser Gly Glu Ile Pro Asp Val Phe Asp Glu Leu Pro Gln Leu Lys Phe
260 265 270

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

Ala Asn Ser Pro Ser Leu Asn Leu Leu Asn Leu Arg Asn Asn Ser Leu
290 295 300

Ser Gly Arg Leu Met Leu Asn Cys Thr Ala Met Ile Ala Leu Asn Ser
305 310 315 320

Leu Asp Leu Gly Thr Asn Arg Phe Asn Gly Arg Leu Pro Glu Asn Leu
325 330 335

Pro Asp Cys Lys Arg Leu Lys Asn Val Asn Leu Ala Arg Asn Thr Phe
340 345 350

His Gly Gln Val Pro Glu Ser Phe Lys Asn Phe Glu Ser Leu Ser Tyr
355 360 365

Phe Ser Leu Ser Asn Ser Ser Leu Ala Asn Ile Ser Ser Ala Leu Gly
370 375 380

Ile Leu Gln His Cys Lys Asn Leu Thr Thr Leu Val Leu Thr Leu Asn
385 390 395 400

Phe His Gly Glu Ala Leu Pro Asp Asp Ser Ser Leu His Phe Glu Lys
405 410 415

Leu Lys Val Leu Val Val Ala Asn Cys Arg Leu Thr Gly Ser Met Pro
420 425 430

Arg Trp Leu Ser Ser Ser Asn Glu Leu Gln Leu Leu Asp Leu Ser Trp
435 440 445

Asn Arg Leu Thr Gly Ala Ile Pro Ser Trp Ile Gly Asp Phe Lys Ala
450 455 460

Leu Phe Tyr Leu Asp Leu Ser Asn Asn Ser Phe Thr Gly Glu Ile Pro
465 470 475 480

Lys Ser Leu Thr Lys Leu Glu Ser Leu Thr Ser Arg Asn Ile Ser Val
485 490 495

Asn Glu Pro Ser Pro Asp Phe Pro Phe Phe Met Lys Arg Asn Glu Ser
500 505 510

Ala Arg Ala Leu Gln Tyr Asn Gln Ile Phe Gly Phe Pro Pro Thr Ile
515 520 525

Glu Leu Gly His Asn Asn Leu Ser Gly Pro Ile Trp Glu Glu Phe Gly
530 535 540

Asn Leu Lys Lys Leu His Val Phe Asp Leu Lys Trp Asn Ala Leu Ser
545 550 555 560

Gly Ser Ile Pro Ser Ser Leu Ser Gly Met Thr Ser Leu Glu Ala Leu
565 570 575

Asp Leu Ser Asn Asn Arg Leu Ser Gly Ser Ile Pro Val Ser Leu Gln
580 585 590

Gln Leu Ser Phe Leu Ser Lys Phe Ser Val Ala Tyr Asn Asn Leu Ser
595 600 605

Gly Val Ile Pro Ser Gly Gly Gln Phe Gln Thr Phe Pro Asn Ser Ser
610 615 620

Phe Glu Ser Asn His Leu Cys Gly Glu His Arg Phe Pro Cys Ser Glu
625 630 635 640

Gly Thr Glu Ser Ala Leu Ile Lys Arg Ser Arg Arg Ser Arg Gly Gly
645 650 655

Asp Ile Gly Met Ala Ile Gly Ile Ala Phe Gly Ser Val Phe Leu Leu
660 665 670

Thr Leu Leu Ser Leu Ile Val Leu Arg Ala Arg Arg Arg Ser Gly Glu
675 680 685

Val Asp Pro Glu Ile Glu Glu Ser Glu Ser Met Asn Arg Lys Glu Leu
690 695 700

Gly Glu Ile Gly Ser Lys Leu Val Val Leu Phe Gln Ser Asn Asp Lys
705 710 715 720

Glu Leu Ser Tyr Asp Asp Leu Leu Asp Ser Thr Asn Ser Phe Asp Gln
725 730 735

Ala Asn Ile Ile Gly Cys Gly Gly Phe Gly Met Val Tyr Lys Ala Thr
740 745 750

Leu Pro Asp Gly Lys Lys Val Ala Ile Lys Lys Leu Ser Gly Asp Cys
755 760 765

Gly Gln Ile Glu Arg Glu Phe Glu Ala Glu Val Glu Thr Leu Ser Arg
770 775 780

Ala Gln His Pro Asn Leu Val Leu Leu Arg Gly Phe Cys Phe Tyr Lys
785 790 795 800

Asn Asp Arg Leu Leu Ile Tyr Ser Tyr Met Glu Asn Gly Ser Leu Asp
805 810 815

Tyr Trp Leu His Glu Arg Asn Asp Gly Pro Ala Leu Leu Lys Trp Lys
820 825 830

Thr Arg Leu Arg Ile Ala Gln Gly Ala Ala Lys Gly Leu Leu Tyr Leu
835 840 845

His Glu Gly Cys Asp Pro His Ile Leu His Arg Asp Ile Lys Ser Ser
850 855 860

Asn Ile Leu Leu Asp Glu Asn Phe Asn Ser His Leu Ala Asp Phe Gly
865 870 875 880

Leu Ala Arg Leu Met Ser Pro Tyr Glu Thr His Val Ser Thr Asp Leu
885 890 895

Val Gly Thr Leu Gly Tyr Ile Pro Pro Glu Tyr Gly Gln Ala Ser Val
900 905 910

Ala Thr Tyr Lys Gly Asp Val Tyr Ser Phe Gly Val Val Leu Leu Glu
915 920 925

Leu Leu Thr Asp Lys Arg Pro Val Asp Met Cys Lys Pro Lys Gly Cys
930 935 940

Arg Asp Leu Ile Ser Trp Val Val Lys Met Lys His Glu Ser Arg Ala
945 950 955 960

Ser Glu Val Phe Asp Pro Leu Ile Tyr Ser Lys Glu Asn Asp Lys Glu
965 970 975

Met Phe Arg Val Leu Glu Ile Ala Cys Leu Cys Leu Ser Glu Asn Pro
980 985 990

Lys Gln Arg Pro Thr Thr Gln Gln Leu Val Ser Trp Leu Asp Asp Val
995 1000 1005

<210> 31

<211> 1036

<212> PRT

<213> *Arabidopsis thaliana*

<400> 31

Met Val Ile Ile Leu Leu Leu Val Phe Phe Val Gly Ser Ser Val Ser
1 5 10 15

Gln Pro Cys His Pro Asn Asp Leu Ser Ala Leu Arg Glu Leu Ala Gly
20 25 30

Ala Leu Lys Asn Lys Ser Val Thr Glu Ser Trp Leu Asn Gly Ser Arg
35 40 45

Cys Cys Glu Trp Asp Gly Val Phe Cys Glu Gly Ser Asp Val Ser Gly
50 55 60

Arg Val Thr Lys Leu Val Leu Pro Glu Lys Gly Leu Glu Gly Val Ile
65 70 75 80

Ser Lys Ser Leu Gly Glu Leu Thr Glu Leu Arg Val Leu Asp Leu Ser
85 90 95

Arg Asn Gln Leu Lys Gly Glu Val Pro Ala Glu Ile Ser Lys Leu Glu
100 105 110

Gln Leu Gln Val Leu Asp Leu Ser His Asn Leu Leu Ser Gly Ser Val
115 120 125

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

Ser Asn Ser Leu Ser Gly Lys Leu Ser Asp Val Gly Val Phe Pro Gly
145 150 155 160

Leu Val Met Leu Asn Val Ser Asn Asn Leu Phe Glu Gly Glu Ile His
165 170 175

Pro Glu Leu Cys Ser Ser Ser Gly Gly Ile Gln Val Leu Asp Leu Ser
180 185 190

Met Asn Arg Leu Val Gly Asn Leu Asp Gly Leu Tyr Asn Cys Ser Lys
195 200 205

Ser Ile Gln Gln Leu His Ile Asp Ser Asn Arg Leu Thr Gly Gln Leu

210		215		220
Pro Asp Tyr Leu Tyr Ser Ile Arg Glu Leu Glu Gln Leu Ser Leu Ser				
225		230		235
Gly Asn Tyr Leu Ser Gly Glu Leu Ser Lys Asn Leu Ser Asn Leu Ser				
		245		250
Gly Leu Lys Ser Leu Leu Ile Ser Glu Asn Arg Phe Ser Asp Val Ile				
		260		265
Pro Asp Val Phe Gly Asn Leu Thr Gln Leu Glu His Leu Asp Val Ser				
		275		280
Ser Asn Lys Phe Ser Gly Arg Phe Pro Pro Ser Leu Ser Gln Cys Ser				
		290		295
Lys Leu Arg Val Leu Asp Leu Arg Asn Asn Ser Leu Ser Gly Ser Ile				
305		310		315
Asn Leu Asn Phe Thr Gly Phe Thr Asp Leu Cys Val Leu Asp Leu Ala				
		325		330
Ser Asn His Phe Ser Gly Pro Leu Pro Asp Ser Leu Gly His Cys Pro				
		340		345
Lys Met Lys Ile Leu Ser Leu Ala Lys Asn Glu Phe Arg Gly Lys Ile				
		355		360
Pro Asp Thr Phe Lys Asn Leu Gln Ser Leu Leu Phe Leu Ser Leu Ser				
		370		375
Asn Asn Ser Phe Val Asp Phe Ser Glu Thr Met Asn Val Leu Gln His				
385		390		395
Cys Arg Asn Leu Ser Thr Leu Ile Leu Ser Lys Asn Phe Ile Gly Glu				
		405		410
Glu Ile Pro Asn Asn Val Thr Gly Phe Asp Asn Leu Ala Ile Leu Ala				
		420		425
Leu Gly Asn Cys Gly Leu Arg Gly Gln Ile Pro Ser Trp Leu Leu Asn				
		435		440
				445

Cys Lys Lys Leu Glu Val Leu Asp Leu Ser Trp Asn His Phe Tyr Gly
450 455 460

Thr Ile Pro His Trp Ile Gly Lys Met Glu Ser Leu Phe Tyr Ile Asp
465 470 475 480

Phe Ser Asn Asn Thr Leu Thr Gly Ala Ile Pro Val Ala Ile Thr Glu
485 490 495

Leu Lys Asn Leu Ile Arg Leu Asn Gly Thr Ala Ser Gln Met Thr Asp
500 505 510

Ser Ser Gly Ile Pro Leu Tyr Val Lys Arg Asn Lys Ser Ser Asn Gly
515 520 525

Leu Pro Tyr Asn Gln Val Ser Arg Phe Pro Pro Ser Ile Tyr Leu Asn
530 535 540

Asn Asn Arg Leu Asn Gly Thr Ile Leu Pro Glu Ile Gly Arg Leu Lys
545 550 555 560

Glu Leu His Met Leu Asp Leu Ser Arg Asn Asn Phe Thr Gly Thr Ile
565 570 575

Pro Asp Ser Ile Ser Gly Leu Asp Asn Leu Glu Val Leu Asp Leu Ser
580 585 590

Tyr Asn His Leu Tyr Gly Ser Ile Pro Leu Ser Phe Gln Ser Leu Thr
595 600 605

Phe Leu Ser Arg Phe Ser Val Ala Tyr Asn Arg Leu Thr Gly Ala Ile
610 615 620

Pro Ser Gly Gly Gln Phe Tyr Ser Phe Pro His Ser Ser Phe Glu Gly
625 630 635 640

Asn Leu Gly Leu Cys Arg Ala Ile Asp Ser Pro Cys Asp Val Leu Met
645 650 655

Ser Asn Met Leu Asn Pro Lys Gly Ser Ser Arg Arg Asn Asn Asn Gly
660 665 670

Gly Lys Phe Gly Arg Ser Ser Ile Val Val Leu Thr Ile Ser Leu Ala

675					680					685					
Ile	Gly	Ile	Thr	Leu	Leu	Leu	Ser	Val	Ile	Leu	Leu	Arg	Ile	Ser	Arg
	690					695					700				
Lys	Asp	Val	Asp	Asp	Arg	Ile	Asn	Asp	Val	Asp	Glu	Glu	Thr	Ile	Ser
705					710					715					720
Gly	Val	Ser	Lys	Ala	Leu	Gly	Pro	Ser	Lys	Ile	Val	Leu	Phe	His	Ser
				725					730					735	
Cys	Gly	Cys	Lys	Asp	Leu	Ser	Val	Glu	Glu	Leu	Leu	Lys	Ser	Thr	Asn
			740					745					750		
Asn	Phe	Ser	Gln	Ala	Asn	Ile	Ile	Gly	Cys	Gly	Gly	Phe	Gly	Leu	Val
		755					760					765			
Tyr	Lys	Ala	Asn	Phe	Pro	Asp	Gly	Ser	Lys	Ala	Ala	Val	Lys	Arg	Leu
	770					775					780				
Ser	Gly	Asp	Cys	Gly	Gln	Met	Glu	Arg	Glu	Phe	Gln	Ala	Glu	Val	Glu
785					790					795					800
Ala	Leu	Ser	Arg	Ala	Glu	His	Lys	Asn	Leu	Val	Ser	Leu	Gln	Gly	Tyr
				805					810					815	
Cys	Lys	His	Gly	Asn	Asp	Arg	Leu	Leu	Ile	Tyr	Ser	Phe	Met	Glu	Asn
			820					825					830		
Gly	Ser	Leu	Asp	Tyr	Trp	Leu	His	Glu	Arg	Val	Asp	Gly	Asn	Met	Thr
		835					840					845			
Leu	Ile	Trp	Asp	Val	Arg	Leu	Lys	Ile	Ala	Gln	Gly	Ala	Ala	Arg	Gly
	850					855					860				
Leu	Ala	Tyr	Leu	His	Lys	Val	Cys	Glu	Pro	Asn	Val	Ile	His	Arg	Asp
865					870					875					880
Val	Lys	Ser	Ser	Asn	Ile	Leu	Leu	Asp	Glu	Lys	Phe	Glu	Ala	His	Leu
				885					890					895	
Ala	Asp	Phe	Gly	Leu	Ala	Arg	Leu	Leu	Arg	Pro	Tyr	Asp	Thr	His	Val
			900					905					910		

Thr Thr Asp Leu Val Gly Thr Leu Gly Tyr Ile Pro Pro Glu Tyr Ser
915 920 925

Gln Ser Leu Ile Ala Thr Cys Arg Gly Asp Val Tyr Ser Phe Gly Val
930 935 940

Val Leu Leu Glu Leu Val Thr Gly Arg Arg Pro Val Glu Val Cys Lys
945 950 955 960

Gly Lys Ser Cys Arg Asp Leu Val Ser Arg Val Phe Gln Met Lys Ala
965 970 975

Glu Lys Arg Glu Ala Glu Leu Ile Asp Thr Thr Ile Arg Glu Asn Val
980 985 990

Asn Glu Arg Thr Val Leu Glu Met Leu Glu Ile Ala Cys Lys Cys Ile
995 1000 1005

Asp His Glu Pro Arg Arg Arg Pro Leu Ile Glu Glu Val Val Thr
1010 1015 1020

Trp Leu Glu Asp Leu Pro Met Glu Ser Val Gln Gln Gln
1025 1030 1035

<210> 32
<211> 1020
<212> PRT
<213> Daucus carota

<400> 32

Met Gly Val Leu Arg Val Tyr Val Ile Leu Ile Leu Val Gly Phe Cys
1 5 10 15

Val Gln Ile Val Val Val Asn Ser Gln Asn Leu Thr Cys Asn Ser Asn
20 25 30

Asp Leu Lys Ala Leu Glu Gly Phe Met Arg Gly Leu Glu Ser Ser Ile
35 40 45

Asp Gly Trp Lys Trp Asn Glu Ser Ser Ser Phe Ser Ser Asn Cys Cys
50 55 60

Asp Trp Val Gly Ile Ser Cys Lys Ser Ser Val Ser Leu Gly Leu Asp
65 70 75 80

Asp Val Asn Glu Ser Gly Arg Val Val Glu Leu Glu Leu Gly Arg Arg
85 90 95

Lys Leu Ser Gly Lys Leu Ser Glu Ser Val Ala Lys Leu Asp Gln Leu
100 105 110

Lys Val Leu Asn Leu Thr His Asn Ser Leu Ser Gly Ser Ile Ala Ala
115 120 125

Ser Leu Leu Asn Leu Ser Asn Leu Glu Val Leu Asp Leu Ser Ser Asn
130 135 140

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

Val Leu Asn Val Tyr Glu Asn Ser Phe His Gly Leu Ile Pro Ala Ser
165 170 175

Leu Cys Asn Asn Leu Pro Arg Ile Arg Glu Ile Asp Leu Ala Met Asn
180 185 190

Tyr Phe Asp Gly Ser Ile Pro Val Gly Ile Gly Asn Cys Ser Ser Val
195 200 205

Glu Tyr Leu Gly Leu Ala Ser Asn Asn Leu Ser Gly Ser Ile Pro Gln
210 215 220

Glu Leu Phe Gln Leu Ser Asn Leu Ser Val Leu Ala Leu Gln Asn Asn
225 230 235 240

Arg Leu Ser Gly Ala Leu Ser Ser Lys Leu Gly Lys Leu Ser Asn Leu
245 250 255

Gly Arg Leu Asp Ile Ser Ser Asn Lys Phe Ser Gly Lys Ile Pro Asp
260 265 270

Val Phe Leu Glu Leu Asn Lys Leu Trp Tyr Phe Ser Ala Gln Ser Asn
275 280 285

Leu Phe Asn Gly Glu Met Pro Arg Ser Leu Ser Asn Ser Arg Ser Ile
290 295 300

Ser Leu Leu Ser Leu Arg Asn Asn Thr Leu Ser Gly Gln Ile Tyr Leu
305 310 315 320

Asn Cys Ser Ala Met Thr Asn Leu Thr Ser Leu Asp Leu Ala Ser Asn
325 330 335

Ser Phe Ser Gly Ser Ile Pro Ser Asn Leu Pro Asn Cys Leu Arg Leu
340 345 350

Lys Thr Ile Asn Phe Ala Lys Ile Lys Phe Ile Ala Gln Ile Pro Glu
355 360 365

Ser Phe Lys Asn Phe Gln Ser Leu Thr Ser Leu Ser Phe Ser Asn Ser
370 375 380

Ser Ile Gln Asn Ile Ser Ser Ala Leu Glu Ile Leu Gln His Cys Gln
385 390 395 400

Asn Leu Lys Thr Leu Val Leu Thr Leu Asn Phe Gln Lys Glu Glu Leu
405 410 415

Pro Ser Val Pro Ser Leu Gln Phe Lys Asn Leu Lys Val Leu Ile Ile
420 425 430

Ala Ser Cys Gln Leu Arg Gly Thr Val Pro Gln Trp Leu Ser Asn Ser
435 440 445

Pro Ser Leu Gln Leu Leu Asp Leu Ser Trp Asn Gln Leu Ser Gly Thr
450 455 460

Ile Pro Pro Trp Leu Gly Ser Leu Asn Ser Leu Phe Tyr Leu Asp Leu
465 470 475 480

Ser Asn Asn Thr Phe Ile Gly Glu Ile Pro His Ser Leu Thr Ser Leu
485 490 495

Gln Ser Leu Val Ser Lys Glu Asn Ala Val Glu Glu Pro Ser Pro Asp
500 505 510

Phe Pro Phe Phe Lys Lys Lys Asn Thr Asn Ala Gly Gly Leu Gln Tyr
515 520 525

Asn Gln Pro Ser Ser Phe Pro Pro Met Ile Asp Leu Ser Tyr Asn Ser
530 535 540

Leu Asn Gly Ser Ile Trp Pro Glu Phe Gly Asp Leu Arg Gln Leu His
545 550 555 560

Val Leu Asn Leu Lys Asn Asn Asn Leu Ser Gly Asn Ile Pro Ala Asn
565 570 575

Leu Ser Gly Met Thr Ser Leu Glu Val Leu Asp Leu Ser His Asn Asn
580 585 590

Leu Ser Gly Asn Ile Pro Pro Ser Leu Val Lys Leu Ser Phe Leu Ser
595 600 605

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

Val Gln Phe Gln Thr Phe Pro Asn Ser Ser Phe Glu Gly Asn Gln Gly
625 630 635 640

Leu Cys Gly Glu His Ala Ser Pro Cys His Ile Thr Asp Gln Ser Pro
645 650 655

His Gly Ser Ala Val Lys Ser Lys Lys Asn Ile Arg Lys Ile Val Ala
660 665 670

Val Ala Val Gly Thr Gly Leu Gly Thr Val Phe Leu Leu Thr Val Thr
675 680 685

Leu Leu Ile Ile Leu Arg Thr Thr Ser Arg Gly Glu Val Asp Pro Glu
690 695 700

Lys Lys Ala Asp Ala Asp Glu Ile Glu Leu Gly Ser Arg Ser Val Val
705 710 715 720

Leu Phe His Asn Lys Asp Ser Asn Asn Glu Leu Ser Leu Asp Asp Ile
725 730 735

Leu Lys Ser Thr Ser Ser Phe Asn Gln Ala Asn Ile Ile Gly Cys Gly
740 745 750

Gly Phe Gly Leu Val Tyr Lys Ala Thr Leu Pro Asp Gly Thr Lys Val
755 760 765

Ala Ile Lys Arg Leu Ser Gly Asp Thr Gly Gln Met Asp Arg Glu Phe
770 775 780

Gln Ala Glu Val Glu Thr Leu Ser Arg Ala Gln His Pro Asn Leu Val
785 790 795 800

His Leu Leu Gly Tyr Cys Asn Tyr Lys Asn Asp Lys Leu Leu Ile Tyr
805 810 815

Ser Tyr Met Asp Asn Gly Ser Leu Asp Tyr Trp Leu His Glu Lys Val
820 825 830

Asp Gly Pro Pro Ser Leu Asp Trp Lys Thr Arg Leu Arg Ile Ala Arg
835 840 845

Gly Ala Ala Glu Gly Leu Ala Tyr Leu His Gln Ser Cys Glu Pro His
850 855 860

Ile Leu His Arg Asp Ile Lys Ser Ser Asn Ile Leu Leu Ser Asp Thr
865 870 875 880

Phe Val Ala His Leu Ala Asp Phe Gly Leu Ala Arg Leu Ile Leu Pro
885 890 895

Tyr Asp Thr His Val Thr Thr Asp Leu Val Gly Thr Leu Gly Tyr Ile
900 905 910

Pro Pro Glu Tyr Gly Gln Ala Ser Val Ala Thr Tyr Lys Gly Asp Val
915 920 925

Tyr Ser Phe Gly Val Val Leu Leu Glu Leu Leu Thr Gly Arg Arg Pro
930 935 940

Met Asp Val Cys Lys Pro Arg Gly Ser Arg Asp Leu Ile Ser Trp Val
945 950 955 960

Leu Gln Met Lys Thr Glu Lys Arg Glu Ser Glu Ile Phe Asp Pro Phe
965 970 975

Ile Tyr Asp Lys Asp His Ala Glu Glu Met Leu Leu Val Leu Glu Ile
980 985 990

Ala Cys Arg Cys Leu Gly Glu Asn Pro Lys Thr Arg Pro Thr Thr Gln
995 1000 1005

Gln Leu Val Ser Trp Leu Glu Asn Ile Asp Val Ser
1010 1015 1020

<210> 33
<211> 1020
<212> PRT
<213> Vitis vinifera

<400> 33

Met Gly Asp Ser Val Phe Trp Val Leu Thr Val Leu Ile Val Leu Gln
1 5 10 15

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

Ala Val Leu Leu Glu Phe Leu Lys Gly Leu Glu Ser Gly Ile Glu Gly
35 40 45

Trp Ser Glu Asn Ser Ser Ser Ala Cys Cys Gly Trp Thr Gly Val Ser
50 55 60

Cys Asn Ser Ser Ala Phe Leu Gly Leu Ser Asp Glu Glu Asn Ser Asn
65 70 75 80

Arg Val Val Gly Leu Glu Leu Gly Gly Met Arg Leu Ser Gly Lys Val
85 90 95

Pro Glu Ser Leu Gly Lys Leu Asp Gln Leu Arg Thr Leu Asn Leu Ser
100 105 110

Ser Asn Phe Phe Lys Gly Ser Ile Pro Ala Ser Leu Phe His Phe Pro
115 120 125

Lys Leu Glu Ser Leu Leu Leu Lys Ala Asn Tyr Phe Thr Gly Ser Ile
130 135 140

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

Asn Ser Leu Ser Gly Ser Leu Pro Gly Gly Ile Cys Gln Asn Ser Thr
165 170 175

Arg Ile Gln Glu Ile Asn Phe Gly Leu Asn His Phe Ser Gly Ser Ile

180	185	190
Pro Val Gly Phe Gly Asn Cys Ser Trp Leu Glu His Leu Cys Leu Ala		
195	200	205
Ser Asn Leu Leu Thr Gly Ala Leu Pro Glu Asp Leu Phe Glu Leu Arg		
210	215	220
Arg Leu Gly Arg Leu Asp Leu Glu Asp Asn Ser Leu Ser Gly Val Leu		
225	230	235
Asp Ser Arg Ile Gly Asn Leu Ser Ser Leu Val Asp Phe Asp Ile Ser		
	245	250
Leu Asn Gly Leu Gly Gly Val Val Pro Asp Val Phe His Ser Phe Glu		
	260	265
Asn Leu Gln Ser Phe Ser Ala His Ser Asn Asn Phe Thr Gly Gln Ile		
	275	280
Pro Tyr Ser Leu Ala Asn Ser Pro Thr Ile Ser Leu Leu Asn Leu Arg		
	290	300
Asn Asn Ser Leu Ser Gly Ser Ile Asn Ile Asn Cys Ser Val Met Gly		
305	310	315
Asn Leu Ser Ser Leu Ser Leu Ala Ser Asn Gln Phe Thr Gly Ser Ile		
	325	330
Pro Asn Asn Leu Pro Ser Cys Arg Arg Leu Lys Thr Val Asn Leu Ala		
	340	345
Arg Asn Asn Phe Ser Gly Gln Ile Pro Glu Thr Phe Lys Asn Phe His		
	355	360
Ser Leu Ser Tyr Leu Ser Leu Ser Asn Ser Ser Leu Tyr Asn Leu Ser		
	370	375
Ser Ala Leu Gly Ile Leu Gln Gln Cys Arg Asn Leu Ser Thr Leu Val		
385	390	395
Leu Thr Leu Asn Phe His Gly Glu Glu Leu Pro Gly Asp Ser Ser Leu		
	405	410
		415

Gln Phe Glu Met Leu Lys Val Leu Val Ile Ala Asn Cys His Leu Ser
420 425 430

Gly Ser Ile Pro His Trp Leu Arg Asn Ser Thr Gly Leu Gln Leu Leu
435 440 445

Asp Leu Ser Trp Asn His Leu Asn Gly Thr Ile Pro Glu Trp Phe Gly
450 455 460

Asp Phe Val Phe Leu Phe Tyr Leu Asp Leu Ser Asn Asn Ser Phe Thr
465 470 475 480

Gly Glu Ile Pro Lys Asn Ile Thr Gly Leu Gln Gly Leu Ile Ser Arg
485 490 495

Glu Ile Ser Met Glu Glu Pro Ser Ser Asp Phe Pro Leu Phe Ile Lys
500 505 510

Arg Asn Val Ser Gly Arg Gly Leu Gln Tyr Asn Gln Val Gly Ser Leu
515 520 525

Pro Pro Thr Leu Asp Leu Ser Asn Asn His Leu Thr Gly Thr Ile Trp
530 535 540

Pro Glu Phe Gly Asn Leu Lys Lys Leu Asn Val Phe Glu Leu Lys Cys
545 550 555 560

Asn Asn Phe Ser Gly Thr Ile Pro Ser Ser Leu Ser Gly Met Thr Ser
565 570 575

Val Glu Thr Met Asp Leu Ser His Asn Asn Leu Ser Gly Thr Ile Pro
580 585 590

Asp Ser Leu Val Glu Leu Ser Phe Leu Ser Lys Phe Ser Val Ala Tyr
595 600 605

Asn Gln Leu Thr Gly Lys Ile Pro Ser Gly Gly Gln Phe Gln Thr Phe
610 615 620

Ser Asn Ser Ser Phe Glu Gly Asn Ala Gly Leu Cys Gly Asp His Ala
625 630 635 640

Ser Pro Cys Pro Ser Asp Asp Ala Asp Asp Gln Val Pro Leu Gly Ser

645	650	655
Pro His Gly Ser Lys Arg Ser Lys Gly Val Ile Ile Gly Met Ser Val		
660	665	670
Gly Ile Gly Phe Gly Thr Thr Phe Leu Leu Ala Leu Met Cys Leu Ile		
675	680	685
Val Leu Arg Thr Thr Arg Arg Gly Glu Val Asp Pro Glu Lys Glu Glu		
690	695	700
Ala Asp Ala Asn Asp Lys Glu Leu Glu Gln Leu Gly Ser Arg Leu Val		
705	710	715
Val Leu Phe Gln Asn Lys Glu Asn Asn Lys Glu Leu Cys Ile Asp Asp		
725	730	735
Leu Leu Lys Ser Thr Asn Asn Phe Asp Gln Ala Asn Ile Ile Gly Cys		
740	745	750
Gly Gly Phe Gly Leu Val Tyr Arg Ala Thr Leu Pro Asp Gly Arg Lys		
755	760	765
Val Ala Ile Lys Arg Leu Ser Gly Asp Cys Gly Gln Met Glu Arg Glu		
770	775	780
Phe Gln Ala Glu Val Glu Ala Leu Ser Arg Ala Gln His Pro Asn Leu		
785	790	795
Val Leu Leu Gln Gly Tyr Cys Lys Tyr Lys Asn Asp Arg Leu Leu Ile		
805	810	815
Tyr Ser Tyr Met Glu Asn Ser Ser Leu Asp Tyr Trp Leu His Glu Lys		
820	825	830
Leu Asp Gly Pro Ser Ser Leu Asp Trp Asp Thr Arg Leu Gln Ile Ala		
835	840	845
Gln Gly Ala Ala Met Gly Leu Ala Tyr Leu His Gln Ser Cys Glu Pro		
850	855	860
His Ile Leu His Arg Asp Ile Lys Ser Ser Asn Ile Leu Leu Asp Glu		
865	870	875
		880

Lys Phe Glu Ala His Leu Ala Asp Phe Gly Leu Ala Arg Leu Ile Leu
885 890 895

Pro Tyr Asp Thr His Val Thr Thr Asp Leu Val Gly Thr Leu Gly Tyr
900 905 910

Ile Pro Pro Glu Tyr Gly Gln Ala Ser Val Ala Thr Tyr Lys Gly Asp
915 920 925

Val Tyr Ser Phe Gly Val Val Leu Leu Glu Leu Leu Thr Gly Lys Arg
930 935 940

Pro Met Asp Met Cys Lys Pro Arg Gly Cys Arg Asp Leu Ile Ser Trp
945 950 955 960

Val Ile Gln Met Lys Lys Glu Lys Arg Glu Ser Glu Val Phe Asp Pro
965 970 975

Phe Ile Tyr Asp Lys Gln His Asp Lys Glu Leu Leu Arg Val Leu Asp
980 985 990

Ile Ala Cys Leu Cys Leu Ser Glu Cys Pro Lys Ile Arg Pro Ser Thr
995 1000 1005

Glu Gln Leu Val Ser Trp Leu Asn Asn Ile Leu Met
1010 1015 1020

<210> 34
<211> 1047
<212> PRT
<213> Oryza sativa

<400> 34

Met Met Gln Leu Thr Thr Thr Trp Pro Trp Arg Phe Phe Phe Cys Leu
1 5 10 15

Phe Phe His Leu Leu Phe Leu Phe Pro Thr Asn Ser Leu Asn Gln Ser
20 25 30

Tyr Cys Asp Pro Gly Asp Ala Ser Ala Leu Leu Gly Phe Met Gln Gly
35 40 45

Leu Ser Gly Ser Gly Ser Gly Trp Thr Val Pro Asn Ala Thr Ser Glu
50 55 60

Thr Ala Asn Cys Cys Ala Trp Leu Gly Val Lys Cys Asn Asp Gly Gly
65 70 75 80

Arg Val Ile Gly Leu Asp Leu Gln Gly Met Lys Leu Arg Gly Glu Leu
85 90 95

Ala Val Ser Leu Gly Gln Leu Asp Gln Leu Gln Trp Leu Asn Leu Ser
100 105 110

Ser Asn Asn Leu His Gly Ala Val Pro Ala Thr Leu Val Gln Leu Gln
115 120 125

Arg Leu Gln Arg Leu Asp Leu Ser Asp Asn Glu Phe Ser Gly Glu Phe
130 135 140

Pro Thr Asn Val Ser Leu Pro Val Ile Glu Val Phe Asn Ile Ser Leu
145 150 155 160

Asn Ser Phe Lys Glu Gln His Pro Thr Leu His Gly Ser Thr Leu Leu
165 170 175

Ala Met Phe Asp Ala Gly Tyr Asn Met Phe Thr Gly His Ile Asp Thr
180 185 190

Ser Ile Cys Asp Pro Asn Gly Val Ile Arg Val Leu Arg Phe Thr Ser
195 200 205

Asn Leu Leu Ser Gly Glu Phe Pro Ala Gly Phe Gly Asn Cys Thr Lys
210 215 220

Leu Glu Glu Leu Tyr Val Asp Leu Asn Ser Ile Thr Gly Ser Leu Pro
225 230 235 240

Asp Asp Leu Phe Arg Leu Ser Ser Leu Arg Asp Leu Ser Leu Gln Glu
245 250 255

Asn Gln Leu Ser Gly Arg Met Thr Pro Arg Phe Gly Asn Met Ser Ser
260 265 270

Leu Ser Lys Leu Asp Ile Ser Phe Asn Ser Phe Ser Gly Tyr Leu Pro
275 280 285

Asn Val Phe Gly Ser Leu Gly Lys Leu Glu Tyr Phe Ser Ala Gln Ser
290 295 300

Asn Leu Phe Arg Gly Pro Leu Pro Ser Ser Leu Ser His Ser Pro Ser
305 310 315 320

Leu Lys Met Leu Tyr Leu Arg Asn Asn Ser Phe His Gly Gln Ile Asp
325 330 335

Leu Asn Cys Ser Ala Met Ser Gln Leu Ser Ser Leu Asp Leu Gly Thr
340 345 350

Asn Lys Phe Ile Gly Thr Ile Asp Ala Leu Ser Asp Cys His His Leu
355 360 365

Arg Ser Leu Asn Leu Ala Thr Asn Asn Leu Thr Gly Glu Ile Pro Asn
370 375 380

Gly Phe Arg Asn Leu Gln Phe Leu Thr Tyr Ile Ser Leu Ser Asn Asn
385 390 395 400

Ser Phe Thr Asn Val Ser Ser Ala Leu Ser Val Leu Gln Gly Cys Pro
405 410 415

Ser Leu Thr Ser Leu Val Leu Thr Lys Asn Phe Asn Asp Gly Lys Ala
420 425 430

Leu Pro Met Thr Gly Ile Asp Gly Phe His Asn Ile Gln Val Phe Val
435 440 445

Ile Ala Asn Ser His Leu Ser Gly Ser Val Pro Ser Trp Val Ala Asn
450 455 460

Phe Ala Gln Leu Lys Val Leu Asp Leu Ser Trp Asn Lys Leu Ser Gly
465 470 475 480

Asn Ile Pro Ala Trp Ile Gly Asn Leu Glu His Leu Phe Tyr Leu Asp
485 490 495

Leu Ser Asn Asn Thr Leu Ser Gly Gly Ile Pro Asn Ser Leu Thr Ser
500 505 510

Met Lys Gly Leu Leu Thr Cys Asn Ser Ser Gln Gln Ser Thr Glu Thr
515 520 525

Asp Tyr Phe Pro Phe Phe Ile Lys Lys Asn Arg Thr Gly Lys Gly Leu
530 535 540

Arg Tyr Asn Gln Val Ser Ser Phe Pro Pro Ser Leu Ile Leu Ser His
545 550 555 560

Asn Met Leu Ile Gly Pro Ile Leu Pro Gly Phe Gly Asn Leu Lys Asn
565 570 575

Leu His Val Leu Asp Leu Ser Asn Asn His Ile Ser Gly Met Ile Pro
580 585 590

Asp Glu Leu Ser Gly Met Ser Ser Leu Glu Ser Leu Asp Leu Ser His
595 600 605

Asn Asn Leu Thr Gly Ser Ile Pro Ser Ser Leu Thr Lys Leu Asn Phe
610 615 620

Leu Ser Ser Phe Ser Val Ala Phe Asn Asn Leu Thr Gly Ala Ile Pro
625 630 635 640

Leu Gly Gly Gln Phe Ser Thr Phe Thr Gly Ser Ala Tyr Glu Gly Asn
645 650 655

Pro Lys Leu Cys Gly Ile Arg Ser Gly Leu Ala Leu Cys Gln Ser Ser
660 665 670

His Ala Pro Thr Met Ser Val Lys Lys Asn Gly Lys Asn Lys Gly Val
675 680 685

Ile Leu Gly Ile Ala Ile Gly Ile Ala Leu Gly Ala Ala Phe Val Leu
690 695 700

Ser Val Ala Val Val Leu Val Leu Lys Ser Ser Phe Arg Arg Gln Asp
705 710 715 720

Tyr Ile Val Lys Ala Val Ala Asp Thr Thr Glu Ala Leu Glu Leu Ala
725 730 735

Pro Ala Ser Leu Val Leu Leu Phe Gln Asn Lys Asp Asp Gly Lys Ala
740 745 750

Met Thr Ile Gly Asp Ile Leu Lys Ser Thr Asn Asn Phe Asp Gln Ala
755 760 765

Asn Ile Ile Gly Cys Gly Gly Phe Gly Leu Val Tyr Lys Ala Thr Leu
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Pro Asp Gly Ala Thr Ile Ala Ile Lys Arg Leu Ser Gly Asp Phe Gly
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Gln His Pro Asn Leu Val Leu Leu Gln Gly Tyr Cys Arg Ile Gly Asn
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Asp Arg Leu Leu Ile Tyr Ser Tyr Met Glu Asn Gly Ser Leu Asp His
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Trp Leu His Glu Lys Pro Asp Gly Pro Ser Arg Leu Ser Trp Gln Thr
850 855 860

Arg Leu Gln Ile Ala Lys Gly Ala Ala Arg Gly Leu Ala Tyr Leu His
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Leu Ser Cys Gln Pro His Ile Leu His Arg Asp Ile Lys Ser Ser Asn
885 890 895

Ile Leu Leu Asp Glu Asp Phe Glu Ala His Leu Ala Asp Phe Gly Leu
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Ala Arg Leu Ile Cys Pro Tyr Asp Thr His Val Thr Thr Asp Leu Val
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Gly Thr Leu Gly Tyr Ile Pro Pro Glu Tyr Gly Gln Ser Ser Val Ala
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Asn Phe Lys Gly Asp Val Tyr Ser Phe Gly Ile Val Leu Leu Glu Leu
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Glu Leu Val Ser Trp Val Leu His Met Lys Glu Lys Asn Cys Glu Ala
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Glu Val Leu Asp Arg Ala Met Tyr Asp Lys Lys Phe Glu Met Gln Met
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Val Gln Met Ile Asp Ile Ala Cys Leu Cys Ile Ser Glu Ser Pro
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Gly Val Ile Leu Ser Ala Trp Ser Asn Glu Pro Asn Cys Cys Lys Trp
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Asp Gly Val Val Cys Gly Asn Val Ser Thr Gln Ser Arg Val Ile Arg
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Glu Arg Leu Asp Gln Leu Lys Leu Leu Asp Leu Ser His Asn His Leu
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Glu Gly Gly Leu Pro Leu Asp Leu Ser Lys Met Lys Gln Leu Glu Val

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Val Ser Gly Phe Glu Asn Leu Met Ile Phe Ala Leu Gly Asn Cys Gly
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His Lys Asn Leu Val Ser Leu Gln Gly Tyr Cys Gln His Gly Ser Asp
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Arg Leu Leu Ile Tyr Ser Tyr Met Glu Asn Gly Ser Leu Asp Tyr Trp
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Leu His Glu Arg Val Asp Gly Ser Ser Leu Thr Trp Asp Met Arg Leu
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Lys Ile Ala Gln Gly Ala Ala Arg Gly Leu Ala Tyr Leu His Lys Glu
885 890 895

Pro Asn Ile Val His Arg Asp Ile Lys Thr Ser Asn Ile Leu Leu Asn
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Glu Arg Phe Glu Ala His Leu Ala Asp Phe Gly Leu Ser Arg Leu Leu
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Arg Pro Tyr Asp Thr His Val Thr Thr Asp Leu Val Gly Thr Leu Gly
930 935 940

Tyr Ile Pro Pro Glu Tyr Ser Gln Thr Leu Thr Ala Thr Phe Arg Gly
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Asp Val Tyr Ser Phe Gly Val Val Leu Leu Glu Leu Leu Thr Gly Lys
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Arg Pro Val Glu Val Cys Arg Gly Lys Asn Cys Arg Asp Leu Val Ser
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Trp Val Phe Gln Leu Lys Ser Glu Asn Arg Ala Glu Glu Ile Phe Asp
995 1000 1005

Thr Thr Ile Trp Asp Thr Ser Tyr Glu Lys Gln Leu Leu Glu Val
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Leu Ser Ile Ala Cys Gln Cys Ile Val Gln Asp Pro Arg Gln Arg
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 <400> 48
 agaaaagctgg gtgtccacct ccggatcagg gcttgtgatt ctgagta 47

 <210> 49
 <211> 108
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Forward primer_ PSK4PS-PSK_ transgenic line spPSK4-pepPSK

 <400> 49
 aattcatggg taagttcaca accattttca tcatggctct ctttctttgc tctacgctaa 60
 cctacgcaga agagtttcat acggactaca tctacactca ggacgtaa 108

 <210> 50
 <211> 108
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Reverse primer_ PSK4PS-PSK_ transgenic line spPSK4-pepPSK

 <400> 50
 agcttttacgt cctgagtgta gatgtagtcc gtatgaaact cttctgcgta ggtagcgta 60
 gagcaaagaa ggagagccat gatgaaaatg gttgtgaact tacccatg 108

 <210> 51
 <211> 32
 <212> DNA
 <213> artificial sequence

<220>
 <223> Forward primer attB1_ PSK-B1_ transgenic line spPSK4-pepPSK

<400> 51
 aaaaagcagg ctcatgggt aagttcaca cc 32

<210> 52
 <211> 35
 <212> DNA
 <213> artificial sequence

<220>
 <223> Reverse primer attB2_ PSKstop-B2 _ transgenic line spPSK4-pepPSK

<400> 52
 agaaagctgg gtatcacttt acgtcctgag tgtag 35

<210> 53
 <211> 108
 <212> DNA
 <213> artificial sequence

<220>
 <223> Forward primer_ PSK4PS-PSK_ transgenic line spPSK4-pepPSK-HA

<400> 53
 aattcatggg taagttcaca accattttca tcatggctct ctttctttgc tctacgctaa 60
 cctacgcaga agagtttcat acggactaca tctacactca ggacgtaa 108

<210> 54
 <211> 108
 <212> DNA
 <213> artificial sequence

<220>
 <223> Reverse primer_ PSK4PS-PSK_ transgenic line spPSK4-pepPSK-HA

<400> 54
 agctttacgt cctgagtgtg gatgtagtcc gtatgaaact cttctgcgta ggtagcgta 60
 gagcaaagaa ggagagccat gatgaaaatg gttgtgaact tacccatg 108

<210> 55
 <211> 28
 <212> DNA
 <213> artificial sequence

<220>
 <223> Forward primer_ HA-Hind_ transgenic line spPSK4-pepPSK-HA

<400> 55
 ggtaagcttt acccatacga tgttcctg 28

<210> 56
 <211> 30
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Reverse primer_ HA-XhoI _ transgenic line spPSK4-pepPSK-HA

 <400> 56
 gaactcgagt caagcgtaat ctggaacgtc 30

<210> 57
 <211> 32
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Forward primer attB1_ PSK-B1_ transgenic line spPSK4-pepPSK-HA

 <400> 57
 aaaaagcagg cttcatgggt aagttcacao cc 32

<210> 58
 <211> 32
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Reverse primer attB2_ PSK-HAstop-B2 _ transgenic line
 spPSK4-pepPSK-HA

 <400> 58
 agaaagctgg gtgtcaagcg taatctggaa cg 32

<210> 59
 <211> 34
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Primer attB1 for Cppskr1-2 amplification

 <400> 59
 aaaaagcagg cttcatggca agaaaatgtg agac 34

<210> 60
 <211> 39
 <212> DNA
 <213> artificial sequence

 <220>
 <223> Primer attB2 for Cppskr1-2 amplification

<400> 60
agaaagctgg gtggaaccat tataggaagc gtactaatc 39

<210> 61
<211> 35
<212> DNA
<213> artificial sequence

<220>
<223> Primer attB1 for p35s:PSKR1 amplification

<400> 61
aaaaagcagg ctgttcttga aatgcgtggt catcg 35

<210> 62
<211> 36
<212> DNA
<213> artificial sequence

<220>
<223> Primer attB2 for p35s:PSKR1 amplification

<400> 62
agaaagctgg gtctagacat catcaagcca agagac 36

<210> 63
<211> 35
<212> DNA
<213> artificial sequence

<220>
<223> Primer attB1 for p35s:PSKR1:GFP amplification

<400> 63
aaaaagcagg ctttaccatg cgtgttcacg gtttt 35

<210> 64
<211> 35
<212> DNA
<213> artificial sequence

<220>
<223> Primer attB2 for p35s:PSKR1:GFP amplification

<400> 64
agaaagctgg gtagacatca tcaagccaag agact 35

<210> 65
<211> 34
<212> DNA
<213> artificial sequence

<220>
 <223> Primer attB1 for PSKR1pro:GFP:GUS amplification

<400> 65
 aaaaagcagg cttcatggca agaaaatgtg agac 34

<210> 66
 <211> 32
 <212> DNA
 <213> artificial sequence

<220>
 <223> Primer attB2 for PSKR1pro:GFP:GUS amplification

<400> 66
 agaaagctgg gtttcaagaa cagaggaaga ag 32

<210> 67
 <211> 3042
 <212> DNA
 <213> Lycopersicon esculentum

<220>
 <221> misc_feature
 <223> Solanum lycopersicum, SlPSKR1 CDS

<400> 67
 atgggtgtgt tgcaagtttg tgtgatcttt ttgtttcttg ggatttgctt acaagcacia 60
 tctcaaaatc tccagaactt gatatgtaat ccaaaagatt tgaaagcact tgagggtttt 120
 gtgaagagtt tagagacagt tattgatttc tgggatttgg ggaattctac aaattgttgt 180
 aatttggttag gtgttacttg tgattctggg aggggtggtga agttggagct tgggaaaaga 240
 aggttaaatg ggaaactttc tgaatcttta ggtaatttgg atgagctaag aacccttaat 300
 ctatctcaca atttctttta aggacctgtt ccttttacac tgttgcattt gtctaaattg 360
 gaagtattag acttgagcaa taatgatttc tttggattgt ttcctagtag catgaacttg 420
 cctttgcttc aagttttcaa tatacttgat aattcctttg gaggaccagt tcctttgggt 480
 atctgtgaaa attcaactag agtttctggt attaagatgg gggttaatta ttttaattgg 540
 agtcttccag taggaattgg gaattgtggt tcattaaagc ttttttgtgt tggctctaac 600
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 ttagggaagt taacatatat gtcagctcat tcaaataagg tcttttggtta tataccaact 840

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gggttccgtg ggtagttcc tgattatctt cctacttgtc aaagggtgca aactatcaat	1020
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ttgaaaagaa atgtaagtgt tagagggttg cagtataatc agatttttag cttccctcca	1560
aaactggaac taggtaacaa ctttctcact ggagcaattt tgccggaatt tgggaatctg	1620
aaaaggttac atgttttgga tctgaaaagc aacaacttat ctgggacaat accaagtagc	1680
ctgtctggta tggcgagcgt agagaatttg gatctatccc acaacaatct gattggcagc	1740
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agtcgaaaag tagttgatca ggaaaaggag ctggatgctt ctaacaggga actggaggac	2100
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gctcagcatc cgaatctggt tcatcttcaa ggatattgca agtacagaac tgaccggctt	2400
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ggacctgctt tattggactg ggatctgagg cttcaaattg ctcaaggggc tgcaagagga	2520
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aacattcttc ttgacgaaaa tttcgaagct cacttagctg atttcggtct tgcaaggatt 2640
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cctgaatatg gccaaagcctc cgtagctacc tataaagggg acgtttatag ctttgggtgtg 2760
gttcttttgg agcttctaac atgcaaaaga ccgatggatc cgtgcaagcc tagagcaagc 2820
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gatcctctga tatatgacaa gcagcacgca aaggaaatgt tattggttct tgaaatcgct 2940
tgcctttgtt tgcataaatc tcctaaaata aggccttctt cgcagcagtt agttacttgg 3000
ctcgacaaca taaacacacc acctgatgtt catgtgtttt ag 3042

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<210> 68
<211> 529
<212> DNA
<213> Lycopersicon esculentum

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<220>
<221> misc_feature
<223> Solanum lycopersicum, PSK1 precursor = BK000120

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<400> 68
atcctcacia agacaataaa aagaagaatt ttaagcaaaa aaaaaaatc aataaatcaa 60
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aatttcctac acaacaacag ctcgtttatt gccacaatt aattctcaag aatctaattg 180
gattattagt aataatccaa tttcctcaca agtacaagaa gatttcaatg atctcatggg 240
aatagaagaa tgtgaagaaa aagatgaaat ttgtttcaag agaagaatga ctgcagaggc 300
tcatttagat tatattttata ctcaacacia gccaaaacat tgaacaagtt tatattaata 360
ttattttttt tcttaaggat ggtaatttag taatgttctt ttctatactt taaattatag 420
taciaaagta taaaagaaac ttttaatttat taaaacttgt atttcgatgt atcataagat 480
tgtagtacta tgttttgtga gaattataaa gatagccaaa agttttaatt 529

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<210> 69
<211> 90
<212> PRT
<213> Lycopersicon esculentum

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<220>
<221> MISC_FEATURE
<223> Solanum lycopersicum, protein PSK1 precursor = BK000120

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<400> 69

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Met Glu Gln Lys Asn Ile Phe Phe Leu Leu Ser Leu Met Val Leu Leu
1 5 10 15

Leu Ile Ser Tyr Thr Thr Thr Ala Arg Leu Leu Pro Thr Ile Asn Ser
20 25 30

Gln Glu Ser Asn Gly Ile Ile Ser Asn Asn Pro Ile Ser Ser Gln Val
35 40 45

Gln Glu Asp Phe Asn Asp Leu Met Gly Ile Glu Glu Cys Glu Glu Lys
50 55 60

Asp Glu Ile Cys Phe Lys Arg Arg Met Thr Ala Glu Ala His Leu Asp
65 70 75 80

Tyr Ile Tyr Thr Gln His Lys Pro Lys His
85 90

<210> 70
<211> 490
<212> DNA
<213> Lycopersicon esculentum

<220>
<221> misc_feature
<223> Solanum lycopersicum PSK2 precursor = BK000121

<400> 70
taaaattcta attaacatg tctaaagcca ataccagttt tttcttcatt atacttctcc 60
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acattgatca ccaccaggat catgttaggg aatcaaaaca agtagcaaac gaagagagct 180
gcaacggagg gcaggatgaa gaatgtttag aaagaaggaa cttggctgct caccttgact 240
atatctatac caaaatcag aaccctgtaa ctagtttgct atttggtata ttggaagtag 300
atgagacagt tacatatcac acattaaaat taccttactg tacatcagtc ccgttgattt 360
ttcctgtacg ttaaaatgta ttaatagcat ttcctcttcc gtcctagatg atactatctc 420
tgttttgctt tgtatttggc ggtatttcaa ctaggcatat ggtttaatta cgaaataaaa 480
ccttctttgt 490

<210> 71
<211> 83
<212> PRT

<213> Lycopersicon esculentum

<220>

<221> MISC_FEATURE

<223> Solanum lycopersicum Protein PSK2 precursor = BK000121

<400> 71

Met Ser Lys Ala Asn Thr Ser Phe Phe Phe Ile Ile Leu Leu Leu Cys
1 5 10 15

Phe Ala Leu Ser Tyr Ala Ser Arg Pro Ala Pro Ala Phe His Glu Ala
20 25 30

Ser Leu Asn Ile Asp His His Gln Asp His Val Arg Glu Ser Lys Gln
35 40 45

Val Ala Asn Glu Glu Ser Cys Asn Gly Gly Gln Asp Glu Glu Cys Leu
50 55 60

Glu Arg Arg Asn Leu Ala Ala His Leu Asp Tyr Ile Tyr Thr Gln Asn
65 70 75 80

Gln Asn Pro

<210> 72

<211> 505

<212> DNA

<213> Lycopersicon esculentum

<220>

<221> misc_feature

<223> Solanum lycopersicum PSK3 precursor = BK000122

<400> 72

tatgatgaag caaaatgtat attttgtgct acttcttctt gtttccatga tcatttcttc 60

acaagcatct agtcgttttt tagtaaaca cttgcaagtg gaaaaggaag caaaattaac 120

taataaatct agtgatggag actcaattga gaagatgaga agtactaatt taaatagggt 180

gatgggggta gaagaatatt catgtgagga tgaaaatgat caagaatgca ttaagagaag 240

agttcttgta gaagctcact tggattacat ctacactcaa caccataatc acccttaatt 300

atgagagatt attacttata cttatgtata gttcaaggac taattaatat cgaggtaacc 360

agtaaagttg tcttcacgta atcgataggt gatggattcg aacttcggaa acaatcacia 420

atattgtatt gcatgatggt atagattcat ctacattaca tgaggccctt ccctcaatca 480
atcatgtaca aatataattg cttta 505

<210> 73
<211> 98
<212> PRT
<213> Lycopersicon esculentum

<220>
<221> MISC_FEATURE
<223> Solanum lycopersicum Protein PSK3 precursor = BK000122

<400> 73

Met Met Lys Gln Asn Val Tyr Phe Val Leu Leu Leu Leu Val Ser Met
1 5 10 15

Ile Ile Ser Ser Gln Ala Ser Ser Arg Phe Leu Val Asn Asn Leu Gln
20 25 30

Val Glu Lys Glu Ala Lys Leu Thr Asn Lys Ser Ser Asp Gly Asp Ser
35 40 45

Ile Glu Lys Met Arg Ser Thr Asn Leu Asn Arg Leu Met Gly Leu Glu
50 55 60

Glu Tyr Ser Cys Glu Asp Glu Asn Asp Gln Glu Cys Ile Lys Arg Arg
65 70 75 80

Val Leu Val Glu Ala His Leu Asp Tyr Ile Tyr Thr Gln His His Asn
85 90 95

His Pro

<210> 74
<211> 489
<212> DNA
<213> Lycopersicon esculentum

<220>
<221> misc_feature
<223> Solanum lycopersicum PSK4 precursor = BK000123

<400> 74

gtaagcatct agctagagct aaataataag ccatcatgtc taaagcatct gccagctttt 60

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ttttcatcat ctttctcctc tgttttgccc tgtcctatgc tgctcgccct aaccacttt 120
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gtaaggaaga gagttgcaaa ggagtcaagg aagaagaatg tttagaaagg aggactttgg 240
ctgctcatct tgactatatc tatacccaaa atcagaaccc ttgaagaaag tttacgattc 300
ccaaggacca aaatgatcag ttaatttggt ttacaatgat taattgacct aagttaaagc 360
ttaattcatg tttcactaaa gtagtgatag aacgagttag ttatcacata tatttatagt 420
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tttctggtg 489

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<210> 75
<211> 82
<212> PRT
<213> Lycopersicon esculentum

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<220>
<221> MISC_FEATURE
<223> Solanum lycopersicum Protein PSK4 precursor = BK000123
<400> 75

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Met Ser Lys Ala Ser Ala Ser Phe Phe Phe Ile Ile Leu Leu Leu Cys
1           5           10           15

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Phe Ala Leu Ser Tyr Ala Ala Arg Pro Asn Pro Leu Phe His Glu Ala
          20           25           30

```

```

Thr Leu Asn Asn Ile Gln His Gln Asp Val Val Glu Pro Lys Glu Val
          35           40           45

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Gly Lys Glu Glu Ser Cys Lys Gly Val Lys Glu Glu Glu Cys Leu Glu
          50           55           60

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Arg Arg Thr Leu Ala Ala His Leu Asp Tyr Ile Tyr Thr Gln Asn Gln
65           70           75           80

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Asn Pro

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<210> 76
<211> 25
<212> DNA
<213> artificial sequence

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<220>
 <223> primer SlPSKR1-F3 LRR domain specific

 <400> 76
 ggggtgtgttg caagtttgtg tgatc 25

<210> 77
 <211> 26
 <212> DNA
 <213> artificial sequence

<220>
 <223> primer SlPSKR1-R3 LRR domain specific

 <400> 77
 caagtctaac agttgcagtt ttgagc 26

<210> 78
 <211> 35
 <212> DNA
 <213> artificial sequence

<220>
 <223> primer SlPSKR1-M13-F4 LRR domain specific

 <400> 78
 cacgacgttg taaaacgact tacaagcaca atctc 35

<210> 79
 <211> 35
 <212> DNA
 <213> artificial sequence

<220>
 <223> primer SlPSKR1-M13-R2 LRR domain specific

 <400> 79
 ggataacaat ttcacacagg ctgaggaaca actcc 35

<210> 80
 <211> 25
 <212> DNA
 <213> Artificial

<220>
 <223> primer SlPSKR1-F4-2 TM-kinase domain specific

 <400> 80
 gagggcaacc aaggactctg cggtg 25

<210> 81
 <211> 25
 <212> DNA

<213> Artificial
 <220>
 <223> primer S1PSKR1-R6 TM-kinase domain specific
 <400> 81
 gcaggacatc cgctggaaat ataag 25

<210> 82
 <211> 35
 <212> DNA
 <213> Artificial
 <220>
 <223> primer S1PSKR1-M13F5 TM-kinase domain specific
 <400> 82
 cacgacgttg taaaacgacc tgtcgaaatg ccagc 35

<210> 83
 <211> 35
 <212> DNA
 <213> Artificial
 <220>
 <223> primer S1PSKR1-M13-R5 TM-kinase domain specific
 <400> 83
 ggataacaat ttcacacagg ctgaggaaca actcc 35

<210> 84
 <211> 19
 <212> DNA
 <213> Artificial
 <220>
 <223> primer M13F700 adaptor IRD700-labeled universal
 <400> 84
 cacgacgttg taaaacgac 19

<210> 85
 <211> 20
 <212> DNA
 <213> Artificial
 <220>
 <223> primer M13R800 adaptor IRD800-labeled universal
 <400> 85
 ggataacaat ttcacacagg 20

<210> 86

<211> 263
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> misc_feature
<223> ecotype Columbia, Col-0, CDS PSK1 = At1g13590

<400> 86
atgatgaaga cgaaaagtga agtggtgatc tttttcttca ctctagtatt gcttttaagc 60
aggcttcaag tgttatttta agagaagatg gttttgctcc tcctaaacca tctcccacca 120
cacatgagaa agcaagtact aaagggtgaca gagatggagt agagtgcaag aattcagaca 180
gtgaagaaga atgtcttggtg aagaaaacag tagctgctca caccgattac atctatacac 240
aagatttaaaa cctatctcct tga 263

<210> 87
<211> 264
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> misc_feature
<223> ecotype Columbia, Col-0, CDS PSK2 = At2g22860

<400> 87
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atggagaaga agatagaagg aaaattagat gacatgcata tggtagacga aaactgtggt 180
gcagacgacg aagattgctt aatgaggagg actttggtcg ctcatactga ttacatctat 240
accagaaga agaagcatcc ttga 264

<210> 88
<211> 246
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> misc_feature
<223> ecotype Columbia, Col-0, CDS PSK3 = At3g44735

<400> 88
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attcgaagag gaaaagaaga tcaagagata aatccattag tttcagctac atcagtggaa 120

gaggactcag ttaataaatt gatggggatg gaatattgtg gagaaggaga tgaagaatgt 180
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 cattga 246

<210> 89
 <211> 240
 <212> DNA
 <213> Arabidopsis thaliana

<220>
 <221> misc_feature
 <223> ecotype Columbia, Col-0, CDS PSK4 = At3g49780

<400> 89
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 attgaaggag acaaggttga agaagaaagc tgcaacggaa ttggagaaga agaatgtttg 180
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<210> 90
 <211> 234
 <212> DNA
 <213> Arabidopsis thaliana

<220>
 <221> misc_feature
 <223> ecotype Columbia, Col-0, CDS PSK5 = At5g65870

<400> 90
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 ctagaacagg gagaggtaat ctgtgaaggt gttggagaag aagaatgctt cttgatacga 180
 agaacttttag ttgctcacac tgattacatc tacactcaaa accacaatcc ctaa 234

<210> 91
 <211> 3027
 <212> DNA
 <213> Arabidopsis thaliana

<220>
 <221> misc_feature
 <223> CDS PSKR1 = At2g02220 = AtPSKR1

<400> 91

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gacttcatag cacatctcga accaaaacca gatggttgga tcaattcttc ttcttctaca	180
gactgctgca actggaccgg aatcacctgc aattcaaaca acaccggaag agttattaga	240
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<210> 95
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<212> PRT
<213> Oryza sativa

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<223> Protein PSK 1 precursor = Os07g0124100 gene

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 <223> PSK2 precursor = Os11g0149400

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Val Lys Ala Val Ala Ala Val Ala Ala Asp Gln Leu Val Leu Gln Leu
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Glu Gly Asp Thr Gly Asn Gly Asp Glu Val Ser Glu Leu Met Gly Ala
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Ala Glu Glu Glu Ala Ala Ala Cys Glu Glu Gly Lys Asn Asn Asp Glu
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Thr Gln His Lys Asn Lys Pro
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gttctctgag ctaccattg tgtttttttc tagagtggag aaagtatata taaagtttgt 600
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tgttcg 666

<210> 99
<211> 75
<212> PRT
<213> Oryza sativa

<220>
<221> MISC_FEATURE
<223> Protein PSK 3 precursor = Os03g0675600

<400> 99

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Ile Ser Ile Ser His Gly Gly Arg Ile Gly Pro Ile Glu Pro Ser Lys
20 25 30

Ala Ser Ser Lys Val Val Glu Arg Gly Asn Tyr Asp Gly Arg Val Glu
35 40 45

Gly Cys Glu Glu Asp Asp Cys Leu Val Glu Arg Leu Leu Val Ala His
50 55 60

Leu Asp Tyr Ile Tyr Thr Gln Gly Lys His Asn
65 70 75

<210> 100
<211> 646
<212> DNA
<213> Oryza sativa

<220>
<221> misc_feature
<223> PSK 4 precursor = Os07g0124100

<400> 100

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gccgcctcgt cggcgaccgt ggccatggcc ggccggccaa cgccctacgac gtctctcgac 180
gaggaagcgg ctcaggcggc ggcgagtcg gagatcggcg gcgggtgcaa ggaaggggaa 240
ggggaggagg agtgcctcgc gaggaggacg ctgacggcgc acaccgatta catctacacc 300
cagcagcatc acaactaatt aatcttatcg atcaatcaat aatcaatcaa tcaatcagtc 360
gcttcctctt cgatctacca atactagtat tggtatataa ttaaaactgc aaatccgtca 420

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tgcattgcatg gtatgcccatt cgatccatcc atgattatct ctagtttagat gtagtaacaa      480
actgcatgctg cgtgtttgtgc tcatcagttgt taatttttggc cgcccccttg ttgataaaca      540
gttctttgatc gatgagagct agcttttcggtt ttgtttttgat ttgttggttg gttggttgat      600
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<210> 101
<211> 83
<212> PRT
<213> Oryza sativa

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<220>
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<223> Protein PSK 4 precursor = Os07g0124100

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<400> 101

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Met Ala Ala Arg Thr Val Ala Val Ala Ala Ala Leu Ala Val Leu Leu
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Ile Phe Ala Ala Ser Ser Ala Thr Val Ala Met Ala Gly Arg Pro Thr
          20              25              30

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Pro Thr Thr Ser Leu Asp Glu Glu Ala Ala Gln Ala Ala Ala Gln Ser
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Glu Ile Gly Gly Gly Cys Lys Glu Gly Glu Gly Glu Glu Glu Cys Leu
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Ala Arg Arg Thr Leu Thr Ala His Thr Asp Tyr Ile Tyr Thr Gln Gln
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His His Asn

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<210> 102
<211> 984
<212> DNA
<213> Oryza sativa

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<220>
<221> misc_feature
<223> PSK 5 precursor = Os12g0147800

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<400> 102
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aacaagcaga acatattccc tatactcttc ttggtagctg gtgcttggtg ctactaccag 900
cacaaatgtt accagtatgc atgtttcata aagagaaggt ataatggatt ataaatatat 960
aaacttgcac ttgttttctg ccgc 984

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<210> 103
<211> 102
<212> PRT
<213> Oryza sativa

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<220>
<221> MISC_FEATURE
<223> Protein PSK 5 precursor = Os12g0147800

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<400> 103

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Met Arg Pro Thr Gly Arg Arg Ser Ser Pro Pro Val Ala Ala Ala Leu
1           5           10           15

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Ala Leu Leu Leu Leu Leu Val Leu Phe Phe Phe Ser His Cys Ala Ser
          20           25           30

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Ala Ala Arg Pro Leu Pro Ala Ser Ala Ala Ala Glu Leu Val Leu Gln
35           40           45

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Asp Gly Ala Thr Gly Asn Gly Asp Glu Val Ser Glu Leu Met Gly Ala
50 55 60

Ala Glu Glu Glu Ala Ala Gly Leu Cys Glu Glu Gly Asn Glu Glu Cys
65 70 75 80

Val Glu Arg Arg Met Leu Arg Asp Ala His Leu Asp Tyr Ile Tyr Thr
85 90 95

Gln Lys Arg Asn Arg Pro
100

<210> 104
<211> 837
<212> DNA
<213> Oryza sativa

<220>
<221> misc_feature
<223> phytosulfokine family protein = Os03g0232400

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cgggtgtgga atgcatcagg gagaacctga tcaggcagtg gagtgcaagg gaggggaagc 300
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caaagaagaa aggcttgatg aggcaaacgg atatcgatct tctgcagttc atttctgtgg 600
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gacttccggc catcaccttc agatagtaca tggaactttg ggatgtaagc actgtagata 780
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<210> 105
<211> 738

<212> DNA
<213> Oryza sativa

<220>
<221> misc_feature
<223> Phytosulfokine family protein = Os11g0557000

<400> 105
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ttatccaatc attaatgct gctgctgact tctctctgta tgatcaatgg atcaaatgc 660
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<210> 106
<211> 3528
<212> DNA
<213> Oryza sativa

<400> 106
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cccaccctt tctcttttga ttaaattttc ttccacagtg atgtgagata ttgtgtgacg 3480
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<210> 107
<211> 1052
<212> PRT
<213> Oryza sativa

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<220>
<221> MISC_FEATURE
<223> Protein OsPSKR1 = Os02g0629400 = Os02g41890

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<400> 107

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Ser	Leu	Asn	Gln	Ser	Tyr	Cys	Asp	Pro	Gly	Asp	Ala	Ser	Ala	Leu	Leu	
		35					40					45				
Gly	Phe	Met	Gln	Gly	Leu	Ser	Gly	Ser	Gly	Ser	Gly	Trp	Thr	Val	Pro	
	50					55					60					
Asn	Ala	Thr	Ser	Glu	Thr	Ala	Asn	Cys	Cys	Ala	Trp	Leu	Gly	Val	Lys	
65					70					75					80	
Cys	Asn	Asp	Gly	Gly	Arg	Val	Ile	Gly	Leu	Asp	Leu	Gln	Gly	Met	Lys	
				85					90					95		
Leu	Arg	Gly	Glu	Leu	Ala	Val	Ser	Leu	Gly	Gln	Leu	Asp	Gln	Leu	Gln	
			100					105					110			
Trp	Leu	Asn	Leu	Ser	Ser	Asn	Asn	Leu	His	Gly	Ala	Val	Pro	Ala	Thr	
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Leu	Val	Gln	Leu	Gln	Arg	Leu	Gln	Arg	Leu	Asp	Leu	Ser	Asp	Asn	Glu	
	130					135					140					
Phe	Ser	Gly	Glu	Phe	Pro	Thr	Asn	Val	Ser	Leu	Pro	Val	Ile	Glu	Val	
145					150					155					160	
Phe	Asn	Ile	Ser	Leu	Asn	Ser	Phe	Lys	Glu	Gln	His	Pro	Thr	Leu	His	
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Gly	Ser	Thr	Leu	Leu	Ala	Met	Phe	Asp	Ala	Gly	Tyr	Asn	Met	Phe	Thr	
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Gly	His	Ile	Asp	Thr	Ser	Ile	Cys	Asp	Pro	Asn	Gly	Val	Ile	Arg	Val	
	195						200					205				
Leu	Arg	Phe	Thr	Ser	Asn	Leu	Leu	Ser	Gly	Glu	Phe	Pro	Ala	Gly	Phe	
	210					215					220					
Gly	Asn	Cys	Thr	Lys	Leu	Glu	Glu	Leu	Tyr	Val	Asp	Leu	Asn	Ser	Ile	
225					230					235					240	

Thr Gly Ser Leu Pro Asp Asp Leu Phe Arg Leu Ser Ser Leu Arg Asp
245 250 255

Leu Ser Leu Gln Glu Asn Gln Leu Ser Gly Arg Met Thr Pro Arg Phe
260 265 270

Gly Asn Met Ser Ser Leu Ser Lys Leu Asp Ile Ser Phe Asn Ser Phe
275 280 285

Ser Gly Tyr Leu Pro Asn Val Phe Gly Ser Leu Gly Lys Leu Glu Tyr
290 295 300

Phe Ser Ala Gln Ser Asn Leu Phe Arg Gly Pro Leu Pro Ser Ser Leu
305 310 315 320

Ser His Ser Pro Ser Leu Lys Met Leu Tyr Leu Arg Asn Asn Ser Phe
325 330 335

His Gly Gln Ile Asp Leu Asn Cys Ser Ala Met Ser Gln Leu Ser Ser
340 345 350

Leu Asp Leu Gly Thr Asn Lys Phe Ile Gly Thr Ile Asp Ala Leu Ser
355 360 365

Asp Cys His His Leu Arg Ser Leu Asn Leu Ala Thr Asn Asn Leu Thr
370 375 380

Gly Glu Ile Pro Asn Gly Phe Arg Asn Leu Gln Phe Leu Thr Tyr Ile
385 390 395 400

Ser Leu Ser Asn Asn Ser Phe Thr Asn Val Ser Ser Ala Leu Ser Val
405 410 415

Leu Gln Gly Cys Pro Ser Leu Thr Ser Leu Val Leu Thr Lys Asn Phe
420 425 430

Asn Asp Gly Lys Ala Leu Pro Met Thr Gly Ile Asp Gly Phe His Asn
435 440 445

Ile Gln Val Phe Val Ile Ala Asn Ser His Leu Ser Gly Ser Val Pro
450 455 460

Ser Trp Val Ala Asn Phe Ala Gln Leu Lys Val Leu Asp Leu Ser Trp

465		470		475		480									
Asn	Lys	Leu	Ser	Gly	Asn	Ile	Pro	Ala	Trp	Ile	Gly	Asn	Leu	Glu	His
				485					490					495	
Leu	Phe	Tyr	Leu	Asp	Leu	Ser	Asn	Asn	Thr	Leu	Ser	Gly	Gly	Ile	Pro
			500					505					510		
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		515					520					525			
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		580						585					590		
Ser	Gly	Met	Ile	Pro	Asp	Glu	Leu	Ser	Gly	Met	Ser	Ser	Leu	Glu	Ser
		595					600					605			
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	610					615					620				
Thr	Lys	Leu	Asn	Phe	Leu	Ser	Ser	Phe	Ser	Val	Ala	Phe	Asn	Asn	Leu
625					630					635					640
Thr	Gly	Ala	Ile	Pro	Leu	Gly	Gly	Gln	Phe	Ser	Thr	Phe	Thr	Gly	Ser
				645					650					655	
Ala	Tyr	Glu	Gly	Asn	Pro	Lys	Leu	Cys	Gly	Ile	Arg	Ser	Gly	Leu	Ala
			660					665					670		
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Ala Ala Phe Val Leu Ser Val Ala Val Val Leu Val Leu Lys Ser Ser
705 710 715 720

Phe Arg Arg Gln Asp Tyr Ile Val Lys Ala Val Ala Asp Thr Thr Glu
725 730 735

Ala Leu Glu Leu Ala Pro Ala Ser Leu Val Leu Leu Phe Gln Asn Lys
740 745 750

Asp Asp Gly Lys Ala Met Thr Ile Gly Asp Ile Leu Lys Ser Thr Asn
755 760 765

Asn Phe Asp Gln Ala Asn Ile Ile Gly Cys Gly Gly Phe Gly Leu Val
770 775 780

Tyr Lys Ala Thr Leu Pro Asp Gly Ala Thr Ile Ala Ile Lys Arg Leu
785 790 795 800

Ser Gly Asp Phe Gly Gln Met Glu Arg Glu Phe Lys Ala Glu Val Glu
805 810 815

Thr Leu Ser Lys Ala Gln His Pro Asn Leu Val Leu Leu Gln Gly Tyr
820 825 830

Cys Arg Ile Gly Asn Asp Arg Leu Leu Ile Tyr Ser Tyr Met Glu Asn
835 840 845

Gly Ser Leu Asp His Trp Leu His Glu Lys Pro Asp Gly Pro Ser Arg
850 855 860

Leu Ser Trp Gln Thr Arg Leu Gln Ile Ala Lys Gly Ala Ala Arg Gly
865 870 875 880

Leu Ala Tyr Leu His Leu Ser Cys Gln Pro His Ile Leu His Arg Asp
885 890 895

Ile Lys Ser Ser Asn Ile Leu Leu Asp Glu Asp Phe Glu Ala His Leu
900 905 910

Ala Asp Phe Gly Leu Ala Arg Leu Ile Cys Pro Tyr Asp Thr His Val
915 920 925

Thr Thr Asp Leu Val Gly Thr Leu Gly Tyr Ile Pro Pro Glu Tyr Gly

930	935	940
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Val Leu Leu Glu Leu Leu Thr Gly Lys Arg Pro Val Asp Met Cys Lys		
	965	970 975
Pro Lys Gly Ala Arg Glu Leu Val Ser Trp Val Leu His Met Lys Glu		
	980	985 990
Lys Asn Cys Glu Ala Glu Val Leu Asp Arg Ala Met Tyr Asp Lys Lys		
	995	1000 1005
Phe Glu Met Gln Met Val Gln Met Ile Asp Ile Ala Cys Leu Cys		
1010	1015	1020
Ile Ser Glu Ser Pro Lys Leu Arg Pro Leu Thr His Glu Leu Val		
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<210> 108
 <211> 3909
 <212> DNA
 <213> Oryza sativa

<220>
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Thr Gly Val Ser Cys Asp Leu Gly Arg Val Val Ala Leu Asp Leu Ser
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Arg Leu Gly Arg Leu Pro Ser Leu Arg Arg Leu Asp Leu Ser Ala Asn
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Gly Leu Ala Gly Ala Phe Pro Ala Gly Gly Phe Pro Ala Ile Glu Val
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Gly Gly Ile Asn Val Thr Ala Leu Cys Ala Ser Pro Val Lys Val Leu
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185

190

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Gly Ser Leu Pro Lys Asp Leu Tyr Met Met Pro Ala Leu Arg Lys Leu
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Ser Leu Gln Glu Asn Lys Leu Ser Gly Ser Leu Asp Asp Asp Leu Gly
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Gly Asn Ile Pro Asp Val Phe Gly Lys Leu Arg Ser Leu Glu Ser Leu
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Pro Trp Leu Gln Ser Leu Lys Ser Leu Ser Val Leu Asp Ile Ser Trp
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Ser Arg Asp Val Val Ser Trp Val Leu Gln Met Lys Lys Glu Asp Arg
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Glu Thr Glu Val Phe Asp Pro Thr Ile Tyr Asp Lys Glu Asn Glu Ser
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Asn Cys Ser Ala Leu Thr Asn Leu Ser Ser Leu Asp Leu Ala Thr Asn
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Pro Asp Asn Pro Val Leu His Phe Glu Asn Leu Lys Val Leu Val Met
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Ala Asn Cys Lys Leu Thr Gly Ser Ile Pro Gln Trp Leu Ile Gly Ser
435 440 445

Ser Lys Leu Gln Leu Val Asp Leu Ser Trp Asn Arg Leu Thr Gly Ser
450 455 460

Ile Pro Ser Trp Phe Gly Gly Phe Val Asn Leu Phe Tyr Leu Asp Leu
465 470 475 480

Ser Asn Asn Ser Phe Thr Gly Glu Ile Pro Lys Asn Leu Thr Glu Leu

485

490

495

Pro Ser Leu Ile Asn Arg Ser Ile Ser Ile Glu Glu Pro Ser Pro Asp
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Phe Pro Phe Phe Leu Thr Arg Asn Glu Ser Gly Arg Gly Leu Gln Tyr
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Asn Gln Val Trp Ser Phe Pro Ser Thr Leu Ala Leu Ser Asp Asn Phe
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Leu Thr Gly Gln Ile Trp Pro Glu Phe Gly Asn Leu Lys Lys Leu His
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Ile Phe Ala Leu Ser Ser Asn Asn Leu Ser Gly Pro Ile Pro Ser Glu
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Leu Ser Gly Met Thr Ser Leu Glu Thr Leu Asp Leu Ser His Asn Asn
580 585 590

Leu Ser Gly Thr Ile Pro Trp Ser Leu Val Asn Leu Ser Phe Leu Ser
595 600 605

Lys Phe Ser Val Ala Tyr Asn Gln Leu His Gly Lys Ile Pro Thr Gly
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Ser Gln Phe Met Thr Phe Pro Asn Ser Ser Phe Glu Gly Asn His Leu
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Cys Gly Asp His Gly Thr Pro Pro Cys Pro Arg Ser Asp Gln Val Pro
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Pro Glu Ser Ser Gly Lys Ser Gly Arg Asn Lys Val Ala Ile Thr Gly
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Met Ala Val Gly Ile Val Phe Gly Thr Ala Phe Leu Leu Thr Leu Met
675 680 685

Ile Met Ile Val Leu Arg Ala His Asn Arg Gly Glu Val Asp Pro Glu
690 695 700

Lys Val Asp Ala Asp Thr Asn Asp Lys Glu Leu Glu Glu Phe Gly Ser
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Arg Leu Val Val Leu Leu Gln Asn Lys Glu Ser Tyr Lys Asp Leu Ser
725 730 735

Leu Glu Asp Leu Leu Lys Phe Thr Asn Asn Phe Asp Gln Ala Asn Ile
740 745 750

Ile Gly Cys Gly Gly Phe Gly Leu Val Tyr Arg Ala Thr Leu Pro Asp
755 760 765

Gly Arg Lys Leu Ala Ile Lys Arg Leu Ser Gly Asp Ser Gly Gln Met
770 775 780

Asp Arg Glu Phe Arg Ala Glu Val Glu Ala Leu Ser Arg Ala Gln His
785 790 795 800

Pro Asn Leu Val His Leu Gln Gly Phe Cys Met Leu Lys Asn Asp Lys
805 810 815

Leu Leu Ile Tyr Ser Tyr Met Glu Asn Ser Ser Leu Asp Tyr Trp Leu
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His Glu Lys Leu Asp Gly Pro Ser Ser Leu Asp Trp Asp Thr Arg Leu
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Gln Ile Ala Gln Gly Ala Ala Arg Gly Leu Ala Tyr Leu His Gln Ala
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Cys Glu Pro His Ile Val His Arg Asp Ile Lys Ser Ser Asn Ile Leu
865 870 875 880

Leu Asp Glu Asn Phe Val Ala His Leu Ala Asp Phe Gly Leu Ala Arg
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Leu Ile Leu Pro Tyr Asp Thr His Val Thr Thr Asp Leu Val Gly Thr
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Leu Gly Tyr Ile Pro Pro Glu Tyr Gly Gln Ala Ala Val Ala Thr Tyr
915 920 925

Met Gly Asp Val Tyr Ser Phe Gly Val Val Leu Leu Glu Leu Leu Thr
930 935 940

Gly Lys Arg Pro Met Asp Met Cys Lys Pro Lys Gly Ser Arg Asp Leu

945 950 955 960
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 Asn Thr
 1025

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 ggtataagtg aggacatctt tcagctgcaa aaactgaagc ttttgggtct ccaagataac 720

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Asn Asp Leu Arg Ala Leu Gln Glu Phe Met Arg Gly Leu Gln Ser Ser
          35           40           45

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Ile Gln Gly Trp Gly Thr Thr Asn Ser Ser Ser Ser Asp Cys Cys Asn
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Trp Ser Gly Ile Thr Cys Tyr Ser Ser Ser Ser Leu Gly Leu Val Asn
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Asp Ser Val Asn Ser Gly Arg Val Thr Lys Leu Glu Leu Val Arg Gln
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Arg Leu Thr Gly Lys Leu Val Glu Ser Val Gly Ser Leu Asp Gln Leu
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Lys Thr Leu Asn Leu Ser His Asn Phe Leu Lys Asp Ser Leu Pro Phe
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Ser Leu Phe His Leu Pro Lys Leu Glu Val Leu Asp Leu Ser Ser Asn
130 135 140

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

Phe Leu Asp Ile Ser Ser Asn Ser Leu Ser Gly Ser Leu Pro Thr His
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Ile Cys Gln Asn Ser Ser Arg Ile Gln Val Leu Val Leu Ala Val Asn
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Tyr Phe Ser Gly Ile Leu Ser Pro Gly Leu Gly Asn Cys Thr Thr Leu
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Glu His Leu Cys Leu Gly Met Asn Asp Leu Ile Gly Gly Ile Ser Glu
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Glu Arg Leu Asp Ile Ser Ser Asn Asn Phe Ser Gly Thr Ile Pro Asp
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Val Phe Arg Ser Leu Ser Lys Leu Lys Phe Phe Leu Gly His Ser Asn
275 280 285

Tyr Phe Val Gly Arg Ile Pro Ile Ser Leu Ala Asn Ser Pro Ser Leu
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Asn Leu Leu Asn Leu Arg Asn Asn Ser Phe Gly Gly Ile Val Glu Leu
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Asn Cys Ser Ala Met Thr Asn Leu Ser Ser Leu Asp Leu Ala Thr Asn

325

330

335

Ser Phe Ser Gly Asn Val Pro Ser Tyr Leu Pro Ala Cys Lys Asn Leu
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Ser Phe Lys Asn Phe Gln Gly Leu Ser Tyr Leu Ser Leu Ser Asn Cys
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Ser Ile Thr Asn Leu Ser Ser Thr Leu Arg Ile Leu Gln Gln Cys Lys
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Ser Leu Thr Ala Leu Val Leu Thr Leu Asn Phe Gln Gly Glu Ala Leu
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Ala Asn Cys Arg Leu Thr Gly Ser Ile Pro Gln Trp Leu Ser Asn Ser
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Ser Lys Leu Gln Leu Val Asp Leu Ser Trp Asn Asn Leu Ser Gly Thr
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Ile Pro Ser Trp Phe Gly Gly Phe Val Asn Leu Phe Tyr Leu Asp Leu
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Pro Ser Leu Ile Ser Arg Ser Ile Ser Ile Glu Glu Pro Ser Pro Tyr
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Phe Pro Leu Phe Met Arg Arg Asn Glu Ser Gly Arg Gly Leu Gln Tyr
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Leu Thr Gly Pro Ile Trp Pro Glu Phe Gly Asn Leu Thr Lys Leu His
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Ile Phe Glu Leu Lys Ser Asn Phe Leu Ser Gly Thr Ile Pro Gly Glu
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Leu Ser Gly Met Thr Ser Leu Glu Thr Leu Asp Leu Ser His Asn Asn
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Leu Ser Gly Val Ile Pro Trp Ser Leu Val Asp Leu Ser Phe Leu Ser
595 600 605

Lys Phe Ser Val Ala Tyr Asn Gln Leu Arg Gly Lys Ile Pro Thr Gly
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Cys Gly Asp His Gly Thr Pro Pro Cys Pro Lys Ser Asp Gly Leu Pro
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Met Ala Val Gly Ile Val Phe Gly Ala Ala Ser Leu Leu Val Leu Ile
675 680 685

Ile Val Leu Arg Ala His Ser Arg Gly Leu Ile Leu Lys Arg Trp Met
690 695 700

Leu Thr His Asp Lys Glu Ala Glu Glu Leu Asp Pro Arg Leu Met Val
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Leu Leu Gln Ser Thr Glu Asn Tyr Lys Asp Leu Ser Leu Glu Asp Leu
725 730 735

Leu Lys Ser Thr Asn Asn Phe Asp Gln Ala Asn Ile Ile Gly Cys Gly
740 745 750

Gly Phe Gly Ile Val Tyr Arg Ala Thr Leu Pro Asp Gly Arg Lys Leu
755 760 765

Ala Ile Lys Arg Leu Ser Gly Asp Ser Gly Gln Met Asp Arg Glu Phe
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Pro	Tyr	Met	Glu	Asn	Ser	Ser	Leu	Asp	Tyr	Trp	Leu	His	Glu	Lys	Ile
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Asp	Gly	Pro	Ser	Ser	Leu	Asp	Trp	Asp	Ser	Arg	Leu	Gln	Ile	Ala	Gln
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Ile	Leu	His	Arg	Asp	Ile	Lys	Ser	Ser	Asn	Ile	Leu	Leu	Asp	Lys	Asn
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Phe	Lys	Ala	Tyr	Leu	Ala	Asp	Phe	Gly	Leu	Ala	Arg	Leu	Met	Leu	Pro
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Tyr	Asp	Thr	His	Val	Thr	Thr	Asp	Leu	Val	Gly	Thr	Leu	Gly	Tyr	Ile
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Pro	Pro	Glu	Tyr	Gly	Gln	Ala	Ala	Val	Ala	Thr	Tyr	Lys	Gly	Asp	Val
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Tyr	Ser	Phe	Gly	Val	Val	Leu	Leu	Glu	Leu	Leu	Thr	Gly	Arg	Arg	Pro
	930					935					940				
Met	Asp	Met	Cys	Lys	Pro	Lys	Gly	Ser	Gln	Asp	Leu	Ile	Ser	Trp	Val
945					950					955					960
Ile	Gln	Met	Lys	Lys	Glu	Asp	Arg	Glu	Ser	Glu	Val	Phe	Asp	Pro	Phe
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Ala	Cys	Leu	Cys	Leu	Ser	Glu	His	Pro	Lys	Leu	Arg	Pro	Ser	Thr	Glu
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Asp Leu Lys Ala Leu Glu Gly Phe Val Lys Ser Leu Glu Thr Val Ile
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Asp Phe Trp Asp Leu Gly Asn Ser Thr Asn Cys Cys Asn Leu Val Gly
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Val Thr Cys Asp Ser Gly Arg Val Val Lys Leu Glu Leu Gly Lys Arg
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Arg Leu Asn Gly Lys Leu Ser Glu Ser Leu Gly Asn Leu Asp Glu Leu
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Arg Thr Leu Asn Leu Ser His Asn Phe Phe Lys Gly Pro Val Pro Phe
100 105 110

Thr Leu Leu His Leu Ser Lys Leu Glu Val Leu Asp Leu Ser Asn Asn
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Asp Phe Phe Gly Leu Phe Pro Ser Ser Met Asn Leu Pro Leu Leu Gln
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Val Phe Asn Ile Ser Asp Asn Ser Phe Gly Gly Pro Val Pro Leu Gly
145 150 155 160

Ile Cys Glu Asn Ser Thr Arg Val Ser Val Ile Lys Met Gly Val Asn
165 170 175

Tyr Phe Asn Gly Ser Leu Pro Val Gly Ile Gly Asn Cys Gly Ser Leu

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190

Lys Leu Phe Cys Val Gly Ser Asn Leu Leu Ser Gly Ser Leu Pro Asp
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Glu Leu Phe Lys Leu Ser Arg Leu Thr Val Leu Ser Leu Gln Glu Asn
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Arg Phe Ser Gly Gln Leu Ser Ser Gln Ile Gly Asn Leu Ser Ser Leu
 225 230 235 240

Val His Leu Asp Ile Cys Ser Asn Gly Phe Ser Gly Asn Ile Pro Asp
 245 250 255

Val Phe Asp Arg Leu Gly Lys Leu Thr Tyr Leu Ser Ala His Ser Asn
 260 265 270

Arg Phe Phe Gly Asn Ile Pro Thr Ser Leu Ala Asn Ser Gly Thr Val
 275 280 285

Ser Ser Leu Ser Leu Arg Asn Asn Ser Leu Gly Gly Ile Ile Glu Leu
 290 295 300

Asn Cys Ser Ala Met Val Ser Leu Val Ser Leu Asp Leu Ala Thr Asn
 305 310 315 320

Gly Phe Arg Gly Leu Val Pro Asp Tyr Leu Pro Thr Cys Gln Arg Leu
 325 330 335

Gln Thr Ile Asn Leu Ala Arg Asn Ser Phe Thr Gly Gln Leu Pro Glu
 340 345 350

Ser Phe Lys Asn Phe His Ser Leu Ser Ser Leu Ser Val Ser Asn Asn
 355 360 365

Ser Met His Asn Ile Asp Ala Ala Leu Arg Ile Leu Gln His Cys Lys
 370 375 380

Asn Leu Ser Thr Leu Val Leu Thr Leu Asn Phe Arg Asp Glu Glu Leu
 385 390 395 400

Pro Thr Asp Ser Ser Leu Gln Phe Ser Glu Leu Lys Ala Leu Ile Ile
 405 410 415

Ala Asn Cys Arg Leu Thr Gly Val Val Pro Gln Trp Leu Arg Asn Ser
420 425 430

Ser Lys Leu Gln Leu Leu Asp Leu Ser Trp Asn Arg Leu Ser Gly Thr
435 440 445

Leu Pro Pro Trp Ile Gly Asp Phe Gln Phe Leu Phe Tyr Leu Asp Phe
450 455 460

Ser Asn Asn Ser Phe Thr Gly Glu Ile Pro Lys Glu Ile Thr Arg Leu
465 470 475 480

Lys Ser Leu Ile Ser Gly Pro Val Ser Met Asn Glu Pro Ser Pro Asp
485 490 495

Phe Pro Phe Phe Leu Lys Arg Asn Val Ser Val Arg Gly Leu Gln Tyr
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Asn Gln Ile Phe Ser Phe Pro Pro Thr Leu Glu Leu Gly Asn Asn Phe
515 520 525

Leu Thr Gly Ala Ile Leu Pro Glu Phe Gly Asn Leu Lys Arg Leu His
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Val Leu Asp Leu Lys Ser Asn Asn Leu Ser Gly Thr Ile Pro Ser Ser
545 550 555 560

Leu Ser Gly Met Ala Ser Val Glu Asn Leu Asp Leu Ser His Asn Asn
565 570 575

Leu Ile Gly Ser Ile Pro Ser Ser Leu Val Gln Cys Ser Phe Met Ser
580 585 590

Lys Phe Ser Val Ala Tyr Asn Lys Leu Ser Gly Glu Ile Pro Thr Gly
595 600 605

Gly Gln Phe Pro Thr Phe Pro Thr Ser Ser Phe Glu Gly Asn Gln Gly
610 615 620

Leu Cys Gly Glu His Gly Ser Thr Cys Arg Asn Ala Ser Gln Val Pro
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Arg Asp Ser Val Ala Lys Gly Lys Arg Arg Lys Gly Thr Val Ile Gly

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Tyr	Leu	Ile	Val	Val	Arg	Ala	Ser	Ser	Arg	Lys	Val	Val	Asp	Gln	Glu		
		675					680					685					
Lys	Glu	Leu	Asp	Ala	Ser	Asn	Arg	Glu	Leu	Glu	Asp	Leu	Gly	Ser	Ser		
	690					695					700						
Leu	Val	Ile	Phe	Phe	His	Asn	Lys	Glu	Asn	Thr	Lys	Glu	Met	Cys	Leu		
705					710					715					720		
Asp	Asp	Leu	Leu	Lys	Cys	Thr	Asp	Asn	Phe	Asp	Gln	Ser	Asn	Ile	Val		
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Gly	Cys	Gly	Gly	Phe	Gly	Leu	Val	Tyr	Lys	Ala	Ile	Leu	Arg	Asp	Gly		
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Arg	Glu	Phe	Gln	Ala	Glu	Val	Glu	Ser	Leu	Ser	Arg	Ala	Gln	His	Pro		
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Glu	Lys	Val	Asp	Gly	Pro	Ala	Leu	Leu	Asp	Trp	Asp	Leu	Arg	Leu	Gln		
			820					825					830				
Ile	Ala	Gln	Gly	Ala	Ala	Arg	Gly	Leu	Ala	Tyr	Leu	His	Leu	Ala	Cys		
		835					840					845					
Glu	Pro	His	Ile	Leu	His	Arg	Asp	Ile	Lys	Ser	Ser	Asn	Ile	Leu	Leu		
	850					855					860						
Asp	Glu	Asn	Phe	Glu	Ala	His	Leu	Ala	Asp	Phe	Gly	Leu	Ala	Arg	Ile		
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Gly Tyr Ile Pro Pro Glu Tyr Gly Gln Ala Ser Val Ala Thr Tyr Lys
900 905 910

Gly Asp Val Tyr Ser Phe Gly Val Val Leu Leu Glu Leu Leu Thr Cys
915 920 925

Lys Arg Pro Met Asp Pro Cys Lys Pro Arg Ala Ser Arg Asp Leu Ile
930 935 940

Ser Trp Val Ile Gln Met Lys Lys Gln Lys Arg Glu Thr Glu Val Phe
945 950 955 960

Asp Pro Leu Ile Tyr Asp Lys Gln His Ala Lys Glu Met Leu Leu Val
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Leu Glu Ile Ala Cys Leu Cys Leu His Glu Ser Pro Lys Ile Arg Pro
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Asp Val His Val Phe
1010

FIGURE 1

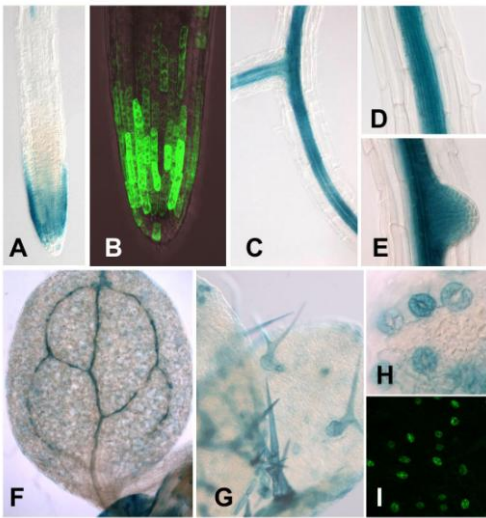
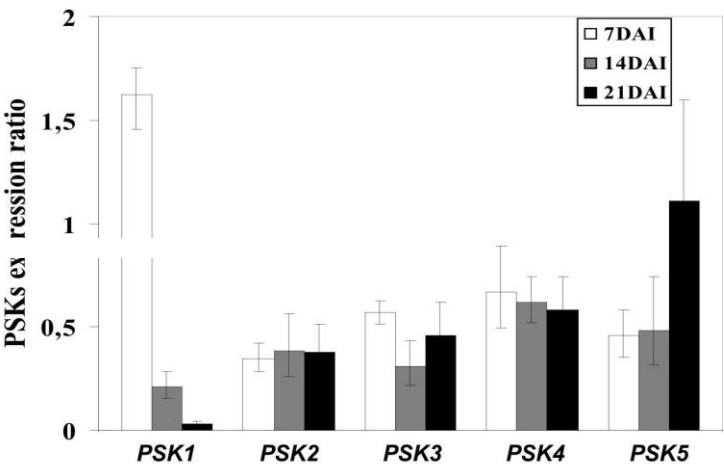


FIGURE 2

2A)

	Nematodes (CATMA)			Oomycetes (Affychip)	
	Log ₂ Ratio in Galls			Log ₂ Ratio in Cotyledons	
	7 dpi	14 dpi	21 dpi	8 + 24 hpi	4 + 6 dpi
At1g13590 PSK1	Not on CATMA Array			nc	nc
At2g22860 PSK2	-1,8	-2	-1,7	-0,9	1,3
At3g44735 PSK3	Not on CATMA Array			nc	nc
At3g49780 PSK4	-1,6	-1,8	-1,5	0,6	2,0
At5g65870 PSK5	Not on CATMA Array			1,0	0,8

2B)



2C)

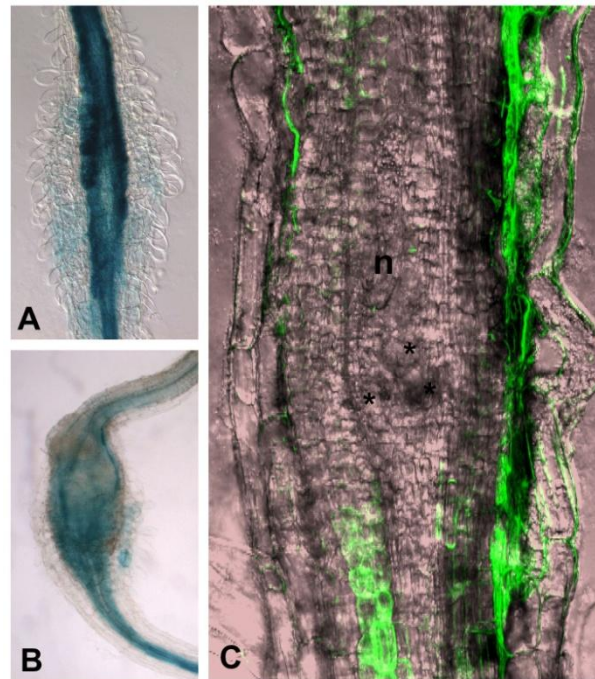


FIGURE 3

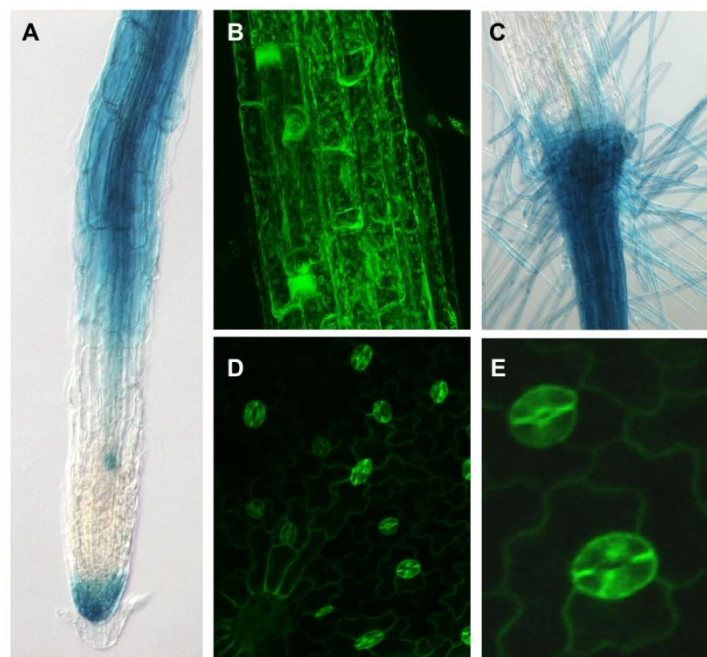
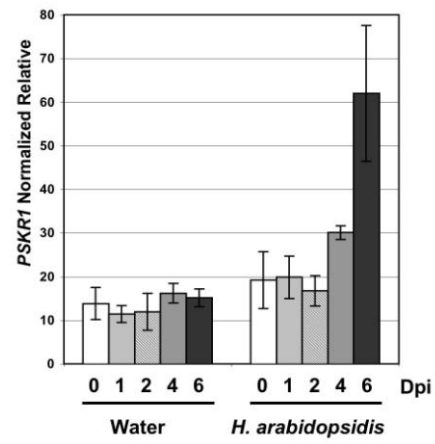


FIGURE 4

4A)



4B)

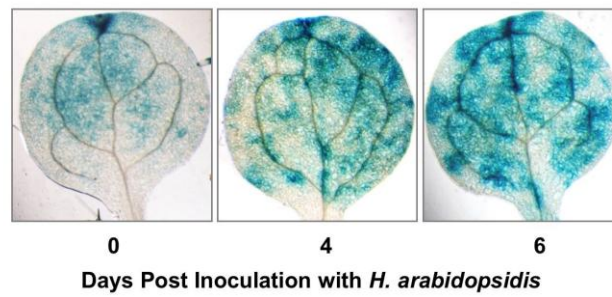
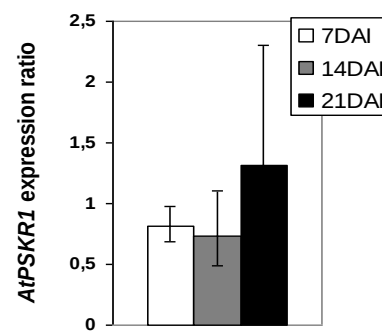


FIGURE 5

5A)



5B)

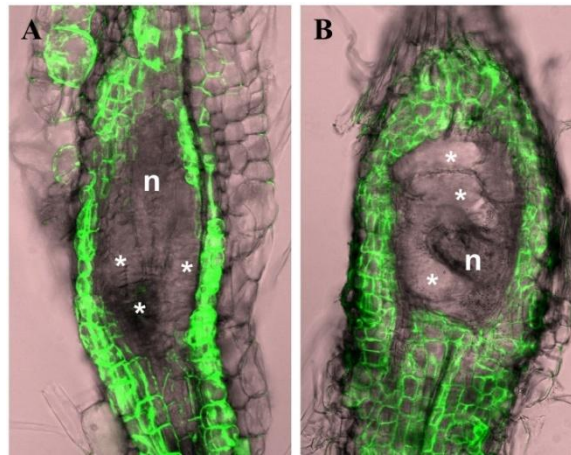
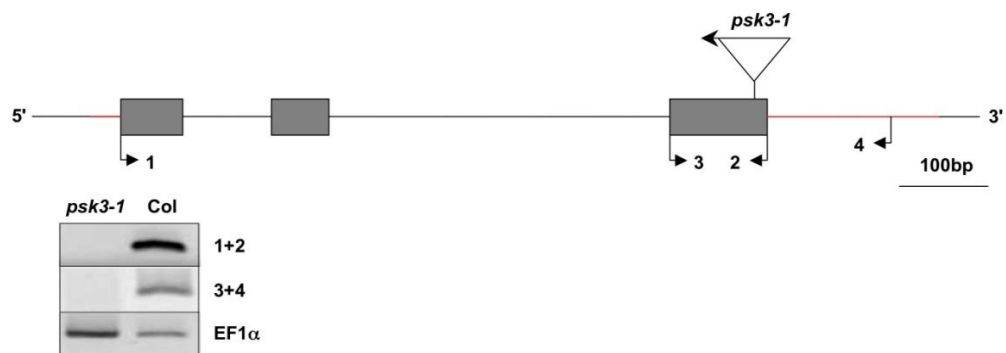


FIGURE 6

6A)



6B)

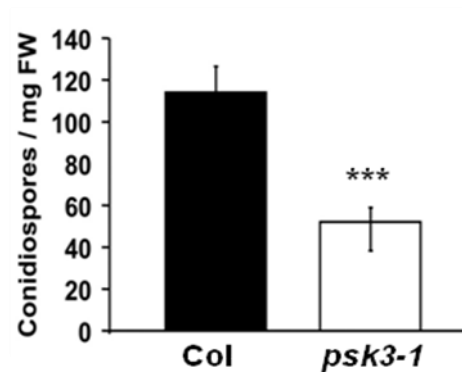
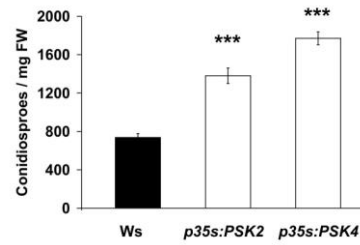
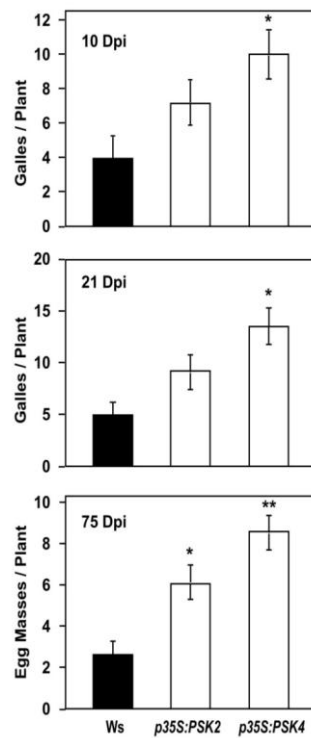


FIGURE 7

7A)



7B)



7C)

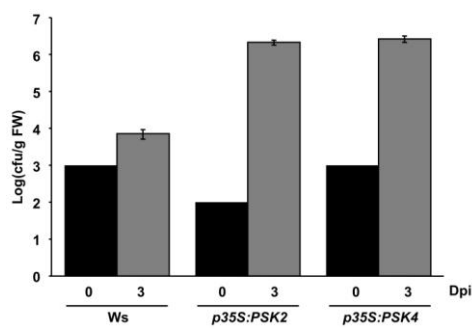
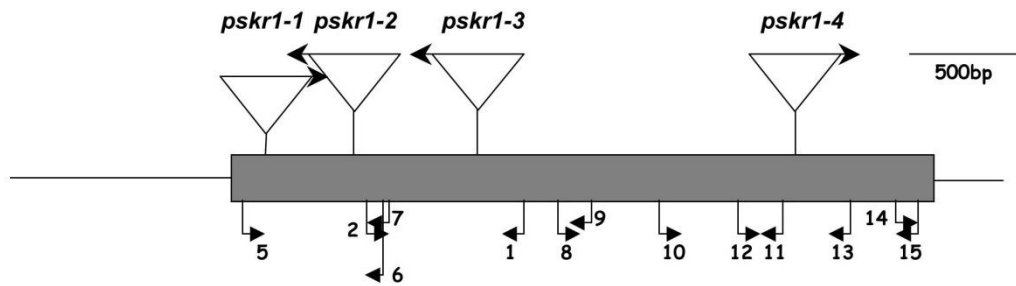
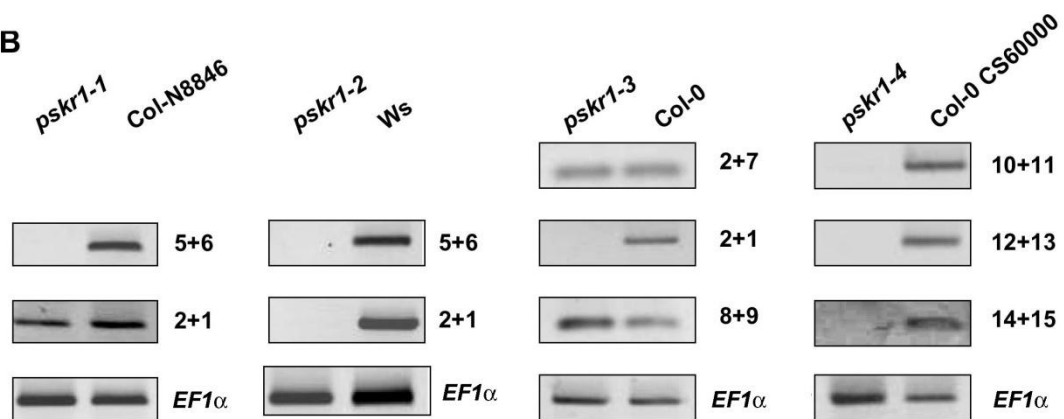


FIGURE 8

A



B



C

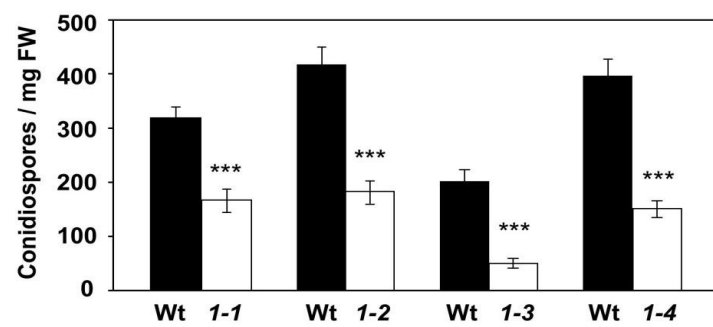


FIGURE 9

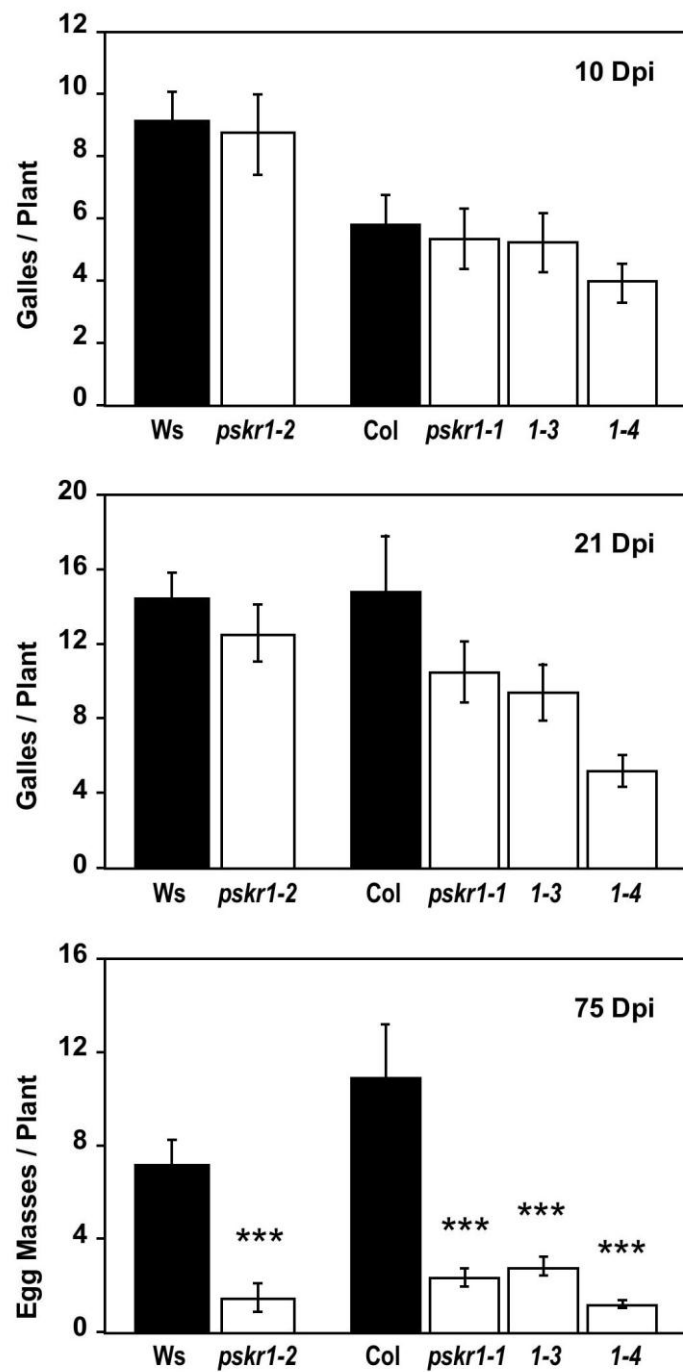


FIGURE 10

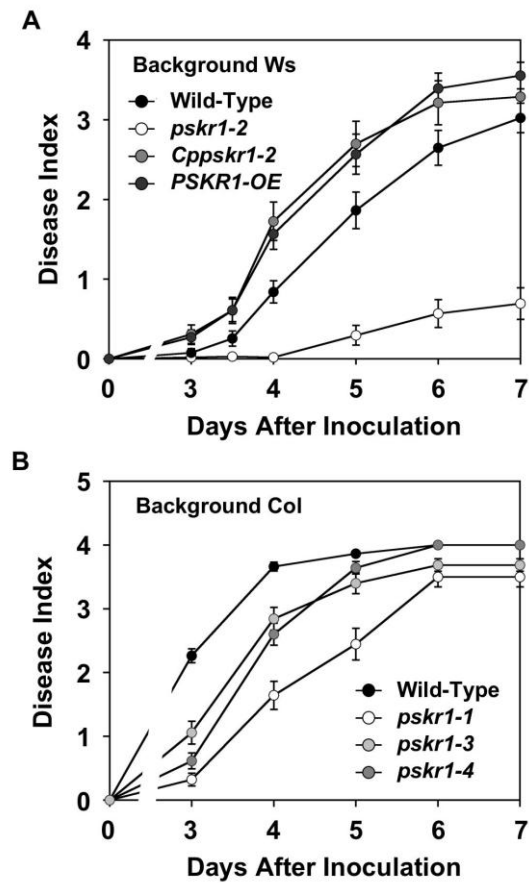


FIGURE 11

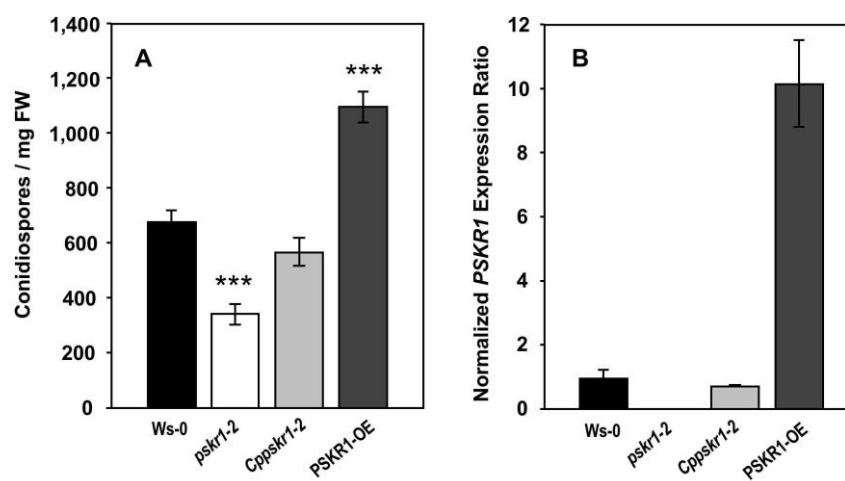


FIGURE 12

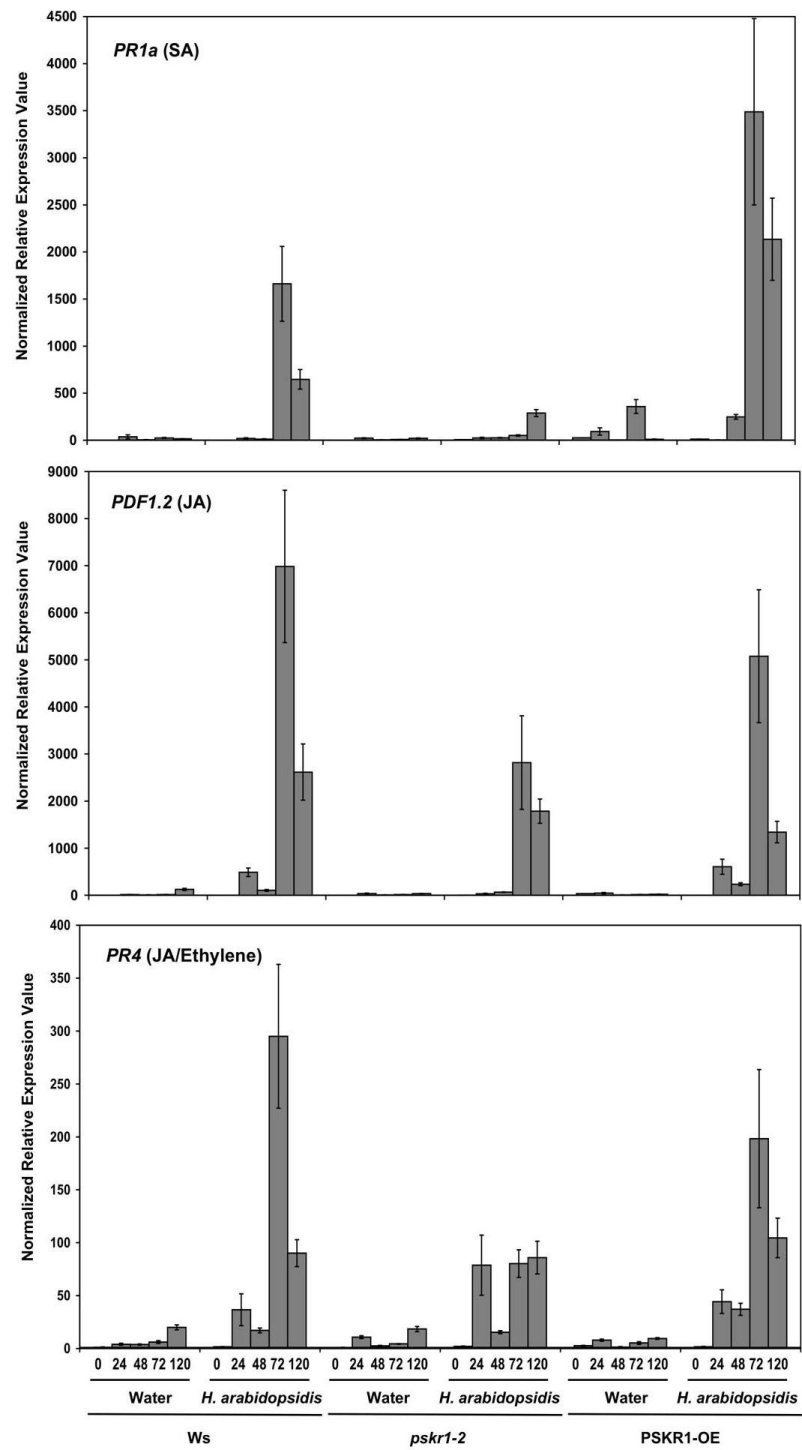


FIGURE 13

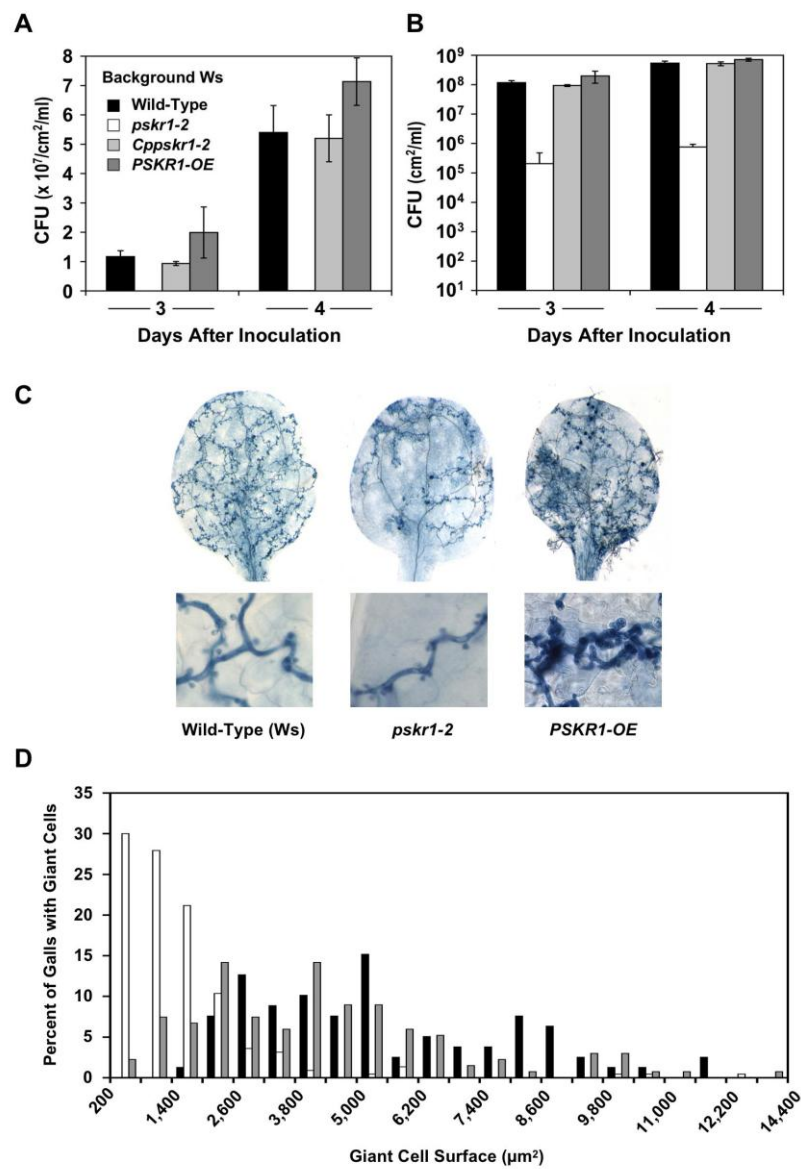


FIGURE 14

