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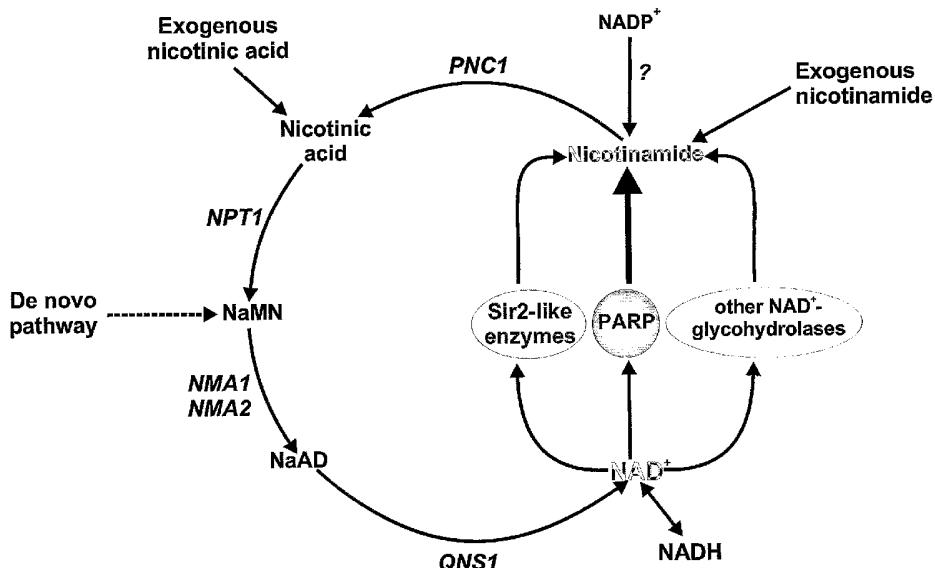
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(54) Title: STRESS RESISTANT PLANTS



NaMN: nicotinic acid mononucleotide
NaAD: desamino-NAD⁺

PNC1: nicotinamidase
NPT1: nicotinate phosphoribosyl transferase
NMA: NAD⁺ pyrophosphorylase
QNS1: NAD⁺ synthetase

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(57) Abstract: Stress tolerance in plants and plant cells is achieved by using nucleotide sequences encoding enzymes involved in the NAD salvage synthesis pathway and/or the NAD *de novo* synthesis pathway e.g. for overexpression in plants.



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STRESS RESISTANT PLANTS

Methods are provided for increasing the stress resistance in plants and plant cells whereby enzymes involved in the NAD salvage synthesis pathway and/or the NAD *de novo* synthesis pathway are expressed in plants.

Background art

Tolerance of plants to adverse growing conditions, including drought, high light intensities, high temperatures, nutrient limitations, saline growing conditions and the like, is a very desired property for crop plants, in view of the never-ending quest to ultimately increase the actual yield of these plants.

Various ways of achieving that goal of improving what is commonly known as the stress resistance or stress tolerance of plants have been described. Since different abiotic stress conditions frequently result in the generation of harmful reactive oxygen species ("ROS") such as superoxides or hydrogen peroxides, initial attempts to improve stress resistance in plants focused on prevention of the generation of the ROS or the removal thereof. Examples of these approaches are overexpression of ROS scavenging enzymes such as catalases, peroxidases, superoxide dismutases etc. or even increasing the amount of ROS scavenging molecules such as ascorbic acid, glutathione etc. These approaches and other attempts to engineer stress tolerant plants are reviewed e.g. in Wang et al. 2003, *Planta* 218:1-14.

Stress tolerance in plant cells and plants can also be achieved by reducing the activity or the level of the endogenous poly-ADP-ribose polymerases (ParP) or poly(ADP-ribose) glycohydrolases (ParG) as described in WO00/04173 and PCT/EP2004/003995, respectively. It is thought that in this way, fatal NAD and ATP depletion in plant cells subject to stress conditions, resulting in traumatic cell death, can be avoided or sufficiently postponed for the stressed cells to survive and acclimate to the stress conditions.

Uchimiya et al. (2002) et al. describe the isolation of a rice gene denoted YK1, as well as use of a chimeric YK1 gene to increase the tolerance of transgenic rice plants harboring that gene to rice blast and several abiotic stresses such as

NaCl, UV-C, submergence, and hydrogen peroxide. (Uchimiya et al., 2002, Molecular breeding 9: 25-31).

Uchimiya et al. further published a poster abstract describing that overexpression of a NAD dependent reductase gene (YK1) in rice cells also promoted the level of NAD(P)(H) through up-regulating NAD synthetase activities, and concluded that this modification in turn generated a pool of redox substances needed for ROS stress resistance (Uchimiya et al. 2003 Keystone symposium on Plant biology: Functions and control of cell death, Snowbird Utah April 10-15, 2003).

NAD synthetase from yeast has been well characterized and is the last enzyme in both the NAD *de novo* synthesis pathway and the NAD salvage pathway (see Figure 1). In the *de novo* pathway, quinolate is the precursor for NAD synthesis and is generated as a product of tryptophan degradation. In the salvage pathway, nicotinamide (which is a degradation product of NAD, generated through the action of various enzymes such as PARP, NAD-dependent deacetylases or other NAD glycohydrolases) is the precursor molecule. In a first step, nicotinamide is deamidated to nicotinic acid by a nicotinamidase. The nicotinic acid is transferred to 5-phosphoribosyl-1-pyrophosphate by the enzyme nicotinate phosphoribosyl transferase to yield nicotinic acid mononucleotide. This compound is shared between the *de novo* and the salvage pathway. Hence, further conversion of this compound by NAD⁺ pyrophosphorylase and NAD synthetase is achieved as in the *de novo* pathway.

In yeast, overexpression of PNC1 (encoding nicotinamidase) has been correlated with life span extension by calorie restriction and low-intensity stress (Anderson et al., 2003 Nature 423: p181-185; Gallo et al., 2004, Molecular and Cellular Biology 24: 1301-1312).

Little is known about the respective enzymes of the NAD biosynthesis pathways in plants. Hunt et al., 2004 describe the use of the available genomic information from Arabidopsis to identify the plant homologues of these enzymes (Hunt et al. , 2004, New Phytologist 163(1): 31-44). The identified DNA sequences have the following Accession numbers: for nicotinamidase: At5g23220; At5g23230 and At3g16190; for nicotinate phosphoribosyltransferase: At4g36940, At2g23420, for

nicotinic acid mononucleotide adenyltransferase: At5g55810 and for NAD synthetase: At1g55090 (all nucleotide sequences are incorporated herein by reference).

Alternative methods for increasing stress tolerance in plants are still required and the embodiments described hereinafter, including the claims, provide such methods and means.

Summary of the invention

In one embodiment of the invention, a method is provided for obtaining a plant with increased stress resistance comprising introducing a chimeric gene into a cells of a plant to obtain transgenic cells whereby the chimeric gene comprises the following operably linked DNA fragments:

- i. A plant-expressible promoter;
- ii. A DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase;
- iii. A 3'end region involved in transcription termination and polyadenylation,

followed by regenerating the transgenic cells to obtain a population of transgenic plants; and selecting a plant from the population of transgenic plants which exhibits increased stress resistance or selecting a plant which exhibits a reduced level of reactive oxygen species or maintains a high level of NADH under stress conditions when compared to a similar non-transgenic plant. The DNA region may code for a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID

No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway such as the nucleotide sequences of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.: 21 or SEQ ID No.:23.

In another embodiment, the invention relates to the chimeric genes as described herein, plant cells comprising these chimeric genes, and plants consisting essentially of plant cells comprising these chimeric genes, and seeds of such plants. These plants and plant cells may be characterized in that they have a lower level of reactive oxygen species under stress conditions than a similar plant not comprising such a chimeric gene.

In yet another embodiment, the invention relates to the use of the described chimeric genes to increase the stress resistance of a plant or to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions.

The invention further provides the use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase, such as a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway, including a DNA sequence

comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23, to increase the stress resistance of a plant or to decrease the level of reactive oxygen species or maintain the level of NADH in a plant or a plant cell under stress conditions.

Brief description of the Figures

Figure 1 is a schematic representation of the NAD salvage pathway and the de novo NAD synthesis pathway as known in baker's yeast (*Saccharomyces cerevisea*)

Figures 2 to 11 are schematic representations of the various T-DNA vectors comprising DNA regions encoding enzymes from the NAD salvage pathway or the NAD *de novo* synthesis pathway under control of plant-expressible control elements. Abbreviations used are: RB: right T-DNA border; 3'35S: transcription termination and polyadenylation signal from CaMV 35S transcript; Cab22L: untranslated leader sequence of the Cab22L transcript; P35S2: CaMV 35S promoter; 3'g7: transcription termination and polyadenylation signal from Agrobacterium tumefaciens T-DNA gene 7; bar: phosphinotricin acetyltransferase coding region; pSSUAra promoter of the Rubisco small subunit transcript from Arabidopsis; LB; left T-DNA border; Sm/Sp: Spectinomycin and streptomycin resistance gene; pVS1ori; origin of VS1 suitable for replication in Agrobacterium; ColE1: origin of replication; NLS: nuclear localization signal; PNC1: DNA region coding for nicotinamidase from *Saccharomyces cereviseae*; npt1: the nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae*; nma1: nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cereviseae*; nma2: nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cereviseae*; qns1: NAD synthetase (QNS1) from *Saccharomyces cerevisea*.

Detailed description

The current invention is based on the finding that DNA sequences encoding plant-functional enzymes from the NAD salvage pathway in yeasts could be used to obtain transgenic plants which were more resistant to stress, particularly abiotic stress, than plants not comprising these DNA sequences. The transgenic plants also exhibited a significantly reduced level of reactive oxygen species ("ROS") and maintained a high level of NADH, when put under stress conditions, compared to control plants

Thus in one embodiment of the invention, a method is provided to obtain a plant with increased stress resistance, whereby the method comprises the steps of

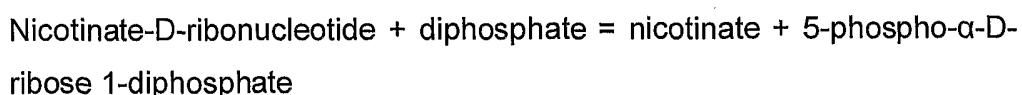
- introducing a stress resistant chimeric gene as herein described into cells of a plant to obtain cells comprising the stress resistant chimeric gene;
- regenerating these cells comprising the stress resistant chimeric gene to obtain a population of plants comprising the stress resistant chimeric gene; and
- selecting a plant from the population of these plants which exhibits increased stress resistance and/or decreased ROS level under stress conditions and/or maintains a high level of NADH, when compared to a similar non-transgenic plant.

The stress resistant chimeric gene thereby comprises a plant-expressible promoter operably linked to a DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase and a 3'end region involved in transcription termination and polyadenylation.

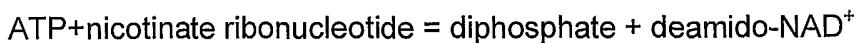
As used herein, "a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway" is an enzyme which when introduced into plants, linked to appropriate control elements such as plant expressible promoter and terminator region, can be transcribed and translated to yield a enzyme of the NAD salvage synthesis pathway functional in plant cells. Included are the enzymes (and encoding genes) from the NAD salvage synthesis, which are obtained from a plant source, but also the enzymes obtained from yeast (*Saccharomyces cerevisiae*) or from other yeasts or fungi. It is thought that the latter proteins may be even more suitable for the methods according to the invention, since these are less likely to be subject to the enzymatic feedback regulation etc. to which similar plant-derived enzymes may be subject.

Enzymes involved in the NAD salvage synthesis pathway comprise the following

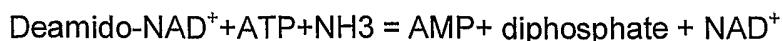
- Nicotinamidase (EC 3.5.1.19) catalyzing the hydrolysis of the amide group of nicotinamide, thereby releasing nicotinate and NH₃. The enzyme is also known as nicotinamide deaminase, nicotinamide amidase, YNDase or nicotinamide amidohydrolase
- Nicotinate phosphoribosyltransferase (EC 2.4.2.11) also known as niacin ribonucleotidase, nicotinic acid mononucleotide glycohydrolase; nicotinic acid mononucleotide pyrophosphorylase; nicotinic acid phosphoribosyltransferase catalyzing the following reaction



- Nicotinate-nucleotide adenylyltransferase, (EC 2.7.7.18) also known as deamido-NAD⁺ pyrophosphorylase; nicotinate mononucleotide adenylyltransferase; deaminonicotinamide adenine dinucleotide pyrophosphorylase; NaMT-ATase; nicotinic acid mononucleotide adenylyltransferase catalyzing the following reaction



- NAD-synthase (EC 6.3.1.5) also known as NAD synthetase; NAD⁺synthase; nicotinamide adenine dinucleotide synthetase; diphosphopyridine nucleotide synthetase, catalyzing the following reaction



In one embodiment of the invention, the coding regions encoding the different enzymes of the NAD salvage pathway comprise a nucleotide sequence encoding proteins with the amino acid sequences as set forth in SEQ ID Nos 2, 4, 6, 8 or 10, such as the nucleotide sequences of SEQ ID Nos 1, 3, 5, 7 or 9.

However, it will be clear that variants of these nucleotide sequences, including insertions, deletions and substitutions thereof may be also be used to the same effect. Equally, homologues to the mentioned nucleotide sequences from species different from *Saccharomyces cerevisea* can be used. These include but are not limited to nucleotide sequences from plants, and nucleotide sequences encoding proteins with the same amino acid sequences, as well as variants of such nucleotide sequences. Examples of the latter are nucleotide sequences encoding a protein with an amino acid sequence as set forth in SEQ ID Nos 12, 14, 16, 18, 20, 22 or 24 such as the nucleotide sequences of SEQ ID Nos 11, 13, 15, 17, 19, 21 or 23.

Variants of the described nucleotide sequence will have a sequence identity which is preferably at least about 80%, or 85 or 90% or 95% with identified nucleotide sequences encoding enzymes from the NAD salvage pathway, such as the ones identified in the sequence listing. Preferably, these variants will encode functional proteins with the same enzymatic activity as the enzymes from the NAD salvage pathway. For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e. a position in an alignment where a residue is

present in one sequence but not in the other, is regarded as a position with non-identical residues. The alignment of the two sequences is performed by the Needleman and Wunsch algorithm (Needleman and Wunsch 1970). The computer-assisted sequence alignment above, can be conveniently performed using standard software program such as GAP which is part of the Wisconsin Package Version 10.1 (Genetics Computer Group, Madison, Wisconsin, USA) using the default scoring matrix with a gap creation penalty of 50 and a gap extension penalty of 3.

Nucleotide sequences homologous to the nucleotide sequences encoding an enzyme from the NAD salvage pathway in yeast, or encoding a homologous enzyme from an organism different than yeast may be identified by *in silico* analysis of genomic data, as described by Hunt et al. (*vide supra*).

Homologous nucleotide sequence may also be identified and isolated by hybridization under stringent conditions using as probes identified nucleotide sequences encoding enzymes from the NAD salvage pathway, such as the ones identified in the sequence listing.

“Stringent hybridization conditions” as used herein means that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt’s solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at approximately 65 °C, preferably twice for about 10 minutes. Other hybridization and wash conditions are well known and are exemplified in Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly chapter 11.

Such variant sequences may also be obtained by DNA amplification using oligonucleotides specific for genes encoding enzymes from the NAD salvage pathway as primers, such as but not limited to oligonucleotides comprising about 20 to about 50 consecutive nucleotides selected from the nucleotide sequences of SEQ ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 or their complement.

The methods of the invention can be used to obtain plants tolerant to different kinds of stress-inducing conditions, particularly abiotic stress conditions including submergence, high light conditions, high UV radiation levels, increased hydrogen peroxide levels, drought conditions, high or low temperatures, increased salinity conditions. The methods of the invention can also be used to reduce the level of ROS in the cells of plants growing under adverse conditions, particularly abiotic stress conditions including submergence, high light conditions, high UV radiation levels, increased hydrogen peroxide levels, drought conditions, high or low temperatures, increased salinity conditions etc. The level of ROS or the level of NADH can be determined using the methods known in the art, including those described in Example 3.

Using the methods described herein, plants may be obtained wherein the level of ROS is equal to or lower than in control plants under non-stressed conditions, such as but not limited to low light. In these plants, under non-stressed conditions, the level of ROS may range from 50% to 100% of the level of control plants under low light conditions, more particularly from about 60% to about 85%. The level of the ROS in these plants under stress conditions is about 50% to 80% of the level of ROS in control plants under stress conditions, corresponding to about 60 to 80% of the level of ROS in control plants under non-stressed conditions. Similarly, the NADH level in these plants is equal to or higher than in control plants under non-stressed conditions, such as but not limited to low light. In these plants, under non-stressed conditions, the level of NADH may range from 100% to 160% of the level of NADH in control plants under low light

conditions, more particularly from about 120% to about 140%. The level of NADH in these plants under stress conditions is about 200 to 300% of the level of NADH in control plants under stress conditions, corresponding to about 100 to 160% of the level of ROS in control plants under non-stressed conditions.

Methods to obtain transgenic plants are not deemed critical for the current invention and any transformation method and regeneration suitable for a particular plant species can be used. Such methods are well known in the art and include *Agrobacterium*-mediated transformation, particle gun delivery, microinjection, electroporation of intact cells, polyethyleneglycol-mediated protoplast transformation, electroporation of protoplasts, liposome-mediated transformation, silicon-whiskers mediated transformation etc. The transformed cells obtained in this way may then be regenerated into mature fertile plants.

The obtained transformed plant can be used in a conventional breeding scheme to produce more transformed plants with the same characteristics or to introduce the chimeric gene according to the invention in other varieties of the same or related plant species, or in hybrid plants. Seeds obtained from the transformed plants contain the chimeric genes of the invention as a stable genomic insert and are also encompassed by the invention.

It will be clear that the different stress resistant chimeric genes described herein, with DNA regions encoding different enzymes from the NAD salvage pathway can be combined within one plant cell or plant, to further enhance the stress tolerance of the plants comprising the chimeric genes. Thus, in one embodiment of the invention, plant cells and plants are provided which comprise at least two stress resistant chimeric genes each comprising a different coding region.

The transgenic plant cells and plant lines according to the invention may further comprise chimeric genes which will reduce the expression of endogenous PARP and/or PARG genes as described in WO 00/04173 and PCT/EP2004/003995 .

These further chimeric genes may be introduced e.g. by crossing the transgenic plant lines of the current invention with transgenic plants containing PARP and/or PARG gene expression reducing chimeric genes. Transgenic plant cells or plant lines may also be obtained by introducing or transforming the chimeric genes of the invention into transgenic plant cells comprising the PARP or PARG gene expression reducing chimeric genes or vice versa.

For the purpose of the invention, the promoter is a plant-expressible promoter. As used herein, the term "plant-expressible promoter" means a DNA sequence which is capable of controlling (initiating) transcription in a plant cell. This includes any promoter of plant origin, but also any promoter of non-plant origin which is capable of directing transcription in a plant cell, i.e., certain promoters of viral or bacterial origin such as the CaMV35S (Harpster et al., 1988 *Mol. Gen. Genet.* **212**, 182-190), the subterranean clover virus promoter No 4 or No 7 (WO9606932), or T-DNA gene promoters but also tissue-specific or organ-specific promoters including but not limited to seed-specific promoters (e.g., WO89/03887), organ-primordia specific promoters (An et al., 1996, *The Plant Cell* **8**, 15-30), stem-specific promoters (Keller et al., 1988, *EMBO J.* **7**, 3625-3633), leaf specific promoters (Hudspeth et al., 1989, *Plant Mol Biol* **12**, 579-589), mesophyl-specific promoters (such as the light-inducible Rubisco promoters), root-specific promoters (Keller et al., 1989, *Genes Devel.* **3**, 1639-1646), tuber-specific promoters (Keil et al., 1989, *EMBO J.* **8**, 1323-1330), vascular tissue specific promoters (Peleman et al., 1989, *Gene* **84**, 359-369), stamen-selective promoters (WO 89/10396, WO 92/13956), dehiscence zone specific promoters (WO 97/13865) and the like.

The chimeric genes of the inventions may also be equipped with a nuclear localization signal ("NLS") functional in plants, operably linked to the DNA region encoding an enzyme of the NAD salvage pathway such as the SV40 NLS.

Having read this document, a person skilled in the art will immediately realize that similar effects with regard to increased stress resistance can be obtained whenever natural variants of plants are obtained wherein the endogenous genes coding for NAD salvage pathway enzymes are more active or expressed at a higher level. Such variant plants can be obtained by subjecting a population of plants to mutagenesis, such as, but not limited to EMS mutagenesis, followed by a screening for an increased activity of any one of the NAD salvage pathway enzymes, or a combination thereof.

It will also be immediately clear that a population of different varieties or cultivars can be screened for increased tolerance to the above mentioned stress conditions in general or particular selected abiotic stresses, followed by a correlation of the increased tolerance to stress conditions with the presence of a particular allele of any of the endogenous genes encoding an enzyme of the NAD salvage pathway enzyme. Such alleles can than be introduced into a plant of interest by crossing, if the species are sexually compatible, or they may be identified using conventional techniques as described herein (including hybridization or PCR amplification) and introduced using recombinant DNA technology. Introduction of particularly desired alleles using breeding techniques may be followed using molecular markers specific for the alleles of interest.

The methods and means described herein are believed to be suitable for all plant cells and plants, both dicotyledonous and monocotyledonous plant cells and plants including but not limited to cotton, Brassica vegetables, oilseed rape, wheat, corn or maize, barley, sunflowers, rice, oats, sugarcane, soybean, vegetables (including chicory, lettuce, tomato), tobacco, potato, sugarbeet, papaya, pineapple, mango, *Arabidopsis thaliana*, but also plants used in horticulture, floriculture or forestry.

As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not

preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a DNA region which is functionally or structurally defined, may comprise additional DNA regions etc.

The following non-limiting Examples describe the construction of chimeric genes to increase stress resistance in plant cells and plants and the use of such genes.

Unless stated otherwise in the Examples, all recombinant DNA techniques are carried out according to standard protocols as described in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel et al. (1994) Current Protocols in Molecular Biology, Current Protocols, USA. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK. Other references for standard molecular biology techniques include Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, NY, Volumes I and II of Brown (1998) Molecular Biology LabFax, Second Edition, Academic Press (UK). Standard materials and methods for polymerase chain reactions can be found in Dieffenbach and Dveksler (1995) PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, and in McPherson et al. (2000) PCR - Basics: From Background to Bench, First Edition, Springer Verlag, Germany.

Throughout the description and Examples, reference is made to the following sequences:

SEQ ID No. 1: nucleotide sequence of the nicotinamidase from *Saccharomyces cerevisiae* (*PNC1*).

SEQ ID No. 2: amino acid sequence of the nicotinamidase from *Saccharomyces cerevisiae* (*PNC1*).

SEQ ID No. 3: nucleotide sequence of the nicotinate phosphoribosyltransferase from *Saccharomyces cerevisiae* (*NPT1*) (complement)

SEQ ID No. 4: amino acid sequence of the nicotinate phosphoribosyltransferase from *Saccharomyces cerevisiae* (*NPT1*)

SEQ ID No. 5: nucleotide sequence of the nicotinic acid mononucleotide adenylyl transferase 1 (*NMA1*) from *Saccharomyces cerevisiae*.

SEQ ID No. 6: amino acid sequence of the nicotinic acid mononucleotide adenylyl transferase 1 (*NMA1*) from *Saccharomyces cerevisiae*

SEQ ID No. 7: nucleotide sequence of the nicotinic acid mononucleotide adenylyl transferase 2 (*NMA2*) from *Saccharomyces cerevisiae*.

SEQ ID No. 8: amino acid sequence of the nicotinic acid mononucleotide adenylyl transferase 2 (*NMA2*) from *Saccharomyces cerevisiae*.

SEQ ID No. 9: nucleotide sequence of the NAD synthetase (*QNS1*) from *Saccharomyces cerevisiae*.

SEQ ID No. 10: amino acid sequence of the NAD synthetase (*QNS1*) from *Saccharomyces cerevisiae*.

SEQ ID No. 11: nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana* (isoform 1).

SEQ ID No. 12: Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana* (isoform 1).

SEQ ID No. 13: nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana* (isoform 2)

SEQ ID No. 14: Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana* (isoform 2).

SEQ ID No. 15: nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana* (isoform 3)

SEQ ID No. 16: Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana* (*isoform 3*).

SEQ ID No. 17: nucleotide sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana* (*isoform 1*).

SEQ ID No. 18: amino acid sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana* (*isoform 1*).

SEQ ID No. 19: nucleotide sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana* (*isoform 2*).

SEQ ID No. 20: amino acid sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana* (*isoform 2*).

SEQ ID No. 21: nucleotide sequence of the nicotinic acid mononucleotide adenyl transferase from *Arabidopsis thaliana*.

SEQ ID No. 22: amino acid sequence of the nicotinic acid mononucleotide adenyl transferase from *Arabidopsis thaliana*.

SEQ ID No. 23: nucleotide sequence of the NAD synthetase from *Arabidopsis thaliana*.

SEQ ID No. 24: amino acid sequence of the NAD synthetase from *Arabidopsis thaliana*.

SEQ ID No. 25: nucleotide sequence of T-DNA vector pTVE 467

SEQ ID No. 26: nucleotide sequence of T-DNA vector pTVE 468

SEQ ID No. 27: nucleotide sequence of T-DNA vector pTVE 469

SEQ ID No. 28: nucleotide sequence of T-DNA vector pTVE 470

SEQ ID No. 29: nucleotide sequence of T-DNA vector pTVE 496

SEQ ID No. 30: nucleotide sequence of T-DNA vector pTVE 497

SEQ ID No. 31: nucleotide sequence of T-DNA vector pTVE 500

SEQ ID No. 32: nucleotide sequence of T-DNA vector pTVE 501

SEQ ID No. 33: nucleotide sequence of T-DNA vector pTVE 502

SEQ ID No. 34: nucleotide sequence of T-DNA vector pTVE 503

Examples

Example 1: Assembly of stress resistant chimeric genes and introduction into plants.

pTVE467

To increase the stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower Mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinamidase from *Saccharomyces cereviseae* (SEQ ID NO 1);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE467 (SEQ ID 25). T-DNA vector pTVE467 is schematically represented in Figure 2.

T-DNA vector pTVE467 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1181	534 (C)	PNC1 coding region
1250	1191(C)	cab22 leader
1781	1251(C)	P35S2 promoter
2293	2082 (C)	3'g7 transcription termination signal
2866	2315 (C)	bar coding region

4592	2867 (C)	PSSuAra promoter
4760	4784	Left T-DNA border
6352	5352 (C)	Sm/Sp resistance gene
6875	10645	pVS1origin of replication
10646	11709	ColE1 origin of replication

pTVE468

A similar chimeric gene as present in pTVE467 was constructed, wherein the nicotinamidase was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinamidase from *Saccharomyces cereviseae* (SEQ ID NO 1); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE468 (SEQ ID 26). T-DNA vector pTVE468 is schematically represented in Figure 3.

T-DNA vector pTVE468 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border

521	300 (C)	3'35S: transcription termination signal
1169	534 (C)	PNC1 coding region
1187	1167 (C)	Nuclear localization signal
1268	1209(C)	cab22 leader
1799	1269(C)	P35S2 promoter
2311	2100 (C)	3'g7 transcription termination signal
2884	2333 (C)	bar coding region
4610	2885 (C)	PSSuAra promoter
4778	4802	Left T-DNA border
6370	5370 (C)	Sm/Sp resistance gene
6893	10663	pVS1origin of replication
10664	11727	ColE1 origin of replication

pTVE469

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinate phosphoribosyltransferase from *Saccharomyces cerevisiae* (NPT1; SEQ ID NO 3);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE469 (SEQ ID 27). T-DNA vector pTVE469 is schematically represented in Figure 4.

T-DNA vector pTVE469 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1765	534 (C)	NPT1 coding region
1832	1773(C)	cab22 leader
2363	1833(C)	P35S2 promoter
2875	2664(C)	3'g7 transcription termination signal
3448	2897 (C)	bar coding region
5175	3449 (C)	PSSuAra promoter
5342	5366	Left T-DNA border
6934	5934(C)	Sm/Sp resistance gene
7457	11227	pVS1origin of replication
11228	12291	ColE1 origin of replication

pTVE470

A similar chimeric gene as present in pTVE469 was constructed, wherein the nicotinate phosphoribosyltransferase from *Saccharomyces cerevisiae* was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinate phosphoribosyltransferase from *Saccharomyces cerevisiae* (NPT1; SEQ ID NO 3); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE470 (SEQ ID 28). T-DNA vector pTVE470 is schematically represented in Figure 5.

T-DNA vector pTVE470 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1787	534 (C)	NPT1 coding region
1775	1755 (C)	Nuclear localization signal SV40
1853	1794(C)	cab22 leader
2384	1854(C)	P35S2 promoter
2896	2685 (C)	3'g7 transcription termination signal
3469	2918(C)	bar coding region
5195	3470 (C)	PSSuAra promoter
5363	5387	Left T-DNA border
6955	5955 (C)	Sm/Sp resistance gene
7478	11248	pVS1origin of replication
11249	12312	ColE1 origin of replication

pTVE496

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;

- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cerevisiae* (NMA1; SEQ ID NO 5);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE496 (SEQ ID 29). T-DNA vector pTVE496 is schematically represented in Figure 6.

T-DNA vector pTVE496 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1739	534 (C)	NMA1 coding region
1805	1746(C)	cab22 leader
2336	1806(C)	P35S2 promoter
2848	2637(C)	3'g7 transcription termination signal
3421	2870 (C)	bar coding region
5147	3422 (C)	PSSuAra promoter
5315	5339	Left T-DNA border
6907	5907(C)	Sm/Sp resistance gene
7430	11200	pVS1origin of replication
11201	12264	ColE1 origin of replication

pTVE497

A similar chimeric gene as present in pTVE496 was constructed, wherein the nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cerevisiae* was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cerevisiae* (NMA1; SEQ ID NO 5); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE497 (SEQ ID 30). T-DNA vector pTVE497 is schematically represented in Figure 7.

T-DNA vector pTVE497 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1757	534 (C)	NMA1 coding region
1748	1731 (C)	Nuclear localization signal SV40
1823	1764(C)	cab22 leader
2354	1824(C)	P35S2 promoter
2866	2655 (C)	3'g7 transcription termination signal
3439	2888(C)	bar coding region
5165	3440 (C)	PSSuAra promoter
5333	5357	Left T-DNA border
6925	5925 (C)	Sm/Sp resistance gene

7448	11218	pVS1origin of replication
11219	12282	ColE1 origin of replication

pTVE500

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cerevisiae* (NMA2; SEQ ID No. 7);
- A fragment of the 3' untranslated end from the 35S transcript of CaMV (3' 35S).

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE500 (SEQ ID 31). T-DNA vector pTVE500 is schematically represented in Figure 8.

T-DNA vector pTVE500 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1721	534 (C)	NMA2 coding region
1787	1728(C)	cab22 leader
2318	1788(C)	P35S2 promoter
2830	2619(C)	3'g7 transcription termination signal
3403	2852 (C)	bar coding region
5129	3404 (C)	PSSuAra promoter

5297	5321	Left T-DNA border
6889	5889(C)	Sm/Sp resistance gene
7412	11182	pVS1origin of replication
11183	12246	ColE1 origin of replication

pTVE501

A similar chimeric gene as present in pTVE500 was constructed, wherein the nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cerevisiae* was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cerevisiae* (NMA2; SEQ ID No. 7); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE502 (SEQ ID 32). T-DNA vector pTVE501 is schematically represented in Figure 9.

T-DNA vector pTVE501 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border

521	300 (C)	3'35S: transcription termination signal
1739	534 (C)	NMA2 coding region
1733	1713 (C)	Nuclear localization signal SV40
1805	1746(C)	cab22 leader
2336	1806(C)	P35S2 promoter
2848	2637 (C)	3'g7 transcription termination signal
3421	2870(C)	bar coding region
5165	3440 (C)	PSSuAra promoter
5315	5339	Left T-DNA border
6907	5907 (C)	Sm/Sp resistance gene
7430	11200	pVS1origin of replication
11201	12264	ColE1 origin of replication

pTVE502

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding NAD synthase from *Saccharomyces cerevisiae* (QNS1; SEQ ID No. 9);
- A fragment of the 3' untranslated end from the 35S transcript of CaMV (3' 35S).

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE502 (SEQ ID 33). T-DNA vector pTVE502 is schematically represented in Figure 10.

T-DNA vector pTVE502 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
2678	534 (C)	QNS1 coding region
2744	2685(C)	cab22 leader
3275	2745(C)	P35S2 promoter
3787	3576 (C)	3'g7 transcription termination signal
4360	3809 (C)	bar coding region
6086	4361 (C)	PSSuAra promoter
6254	6278	Left T-DNA border
7846	6846 (C)	Sm/Sp resistance gene
8369	12139	pVS1origin of replication
12140	13203	ColE1 origin of replication

pTVE503

A similar chimeric gene as present in pTVE502 was constructed, wherein the NAD synthase from *Saccharomyces cereviseae* was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding NAD synthase from *Saccharomyces cereviseae* (QNS1; SEQ ID No. ç); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE503 (SEQ ID No. 34). T-DNA vector pTVE503 is schematically represented in Figure 11.

T-DNA vector pTVE503 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
2699	534 (C)	QNS1 coding region
2690	2670(C)	Nuclear localization signal SV40
2765	2706(C)	cab22 leader
3296	2766(C)	P35S2 promoter
3808	3597 (C)	3'g7 transcription termination signal
4381	3830 (C)	bar coding region
6107	4382 (C)	PSSuAra promoter
6275	6299	Left T-DNA border
7867	6867 (C)	Sm/Sp resistance gene
8390	12610	pVS1origin of replication
12161	13224	ColE1 origin of replication

The T-DNA vectors were introduced into Agrobacterium strains comprising a helper Ti-plasmid using conventional methods. The chimeric genes were introduced into Arabidopsis plants by Agrobacterium mediated transformation as described in the art.

Example 2. Analysis of transgenic Arabidopsis lines comprising the chimeric genes described in Example 1.

Seed of transgenic Arabidopsis lines (T1-generation) expressing the yeast genes of the NAD-salvage pathway, obtained as described in Example 1 were germinated and grown on medium containing 15 mg L⁻¹ phosphinotricin (PPT). Arabidopsis thaliana cv Col-0 was used as a control.

All plants were subjected to high light stress. Two week old plants grown at 30 µEinstein m⁻² sec⁻¹ were transferred to 250 µEinstein m⁻² sec⁻¹ (high light) for 6 hours, followed by 8 hours in the dark and again 8 hours high light.

After this treatment, NADH content and superoxide radicals content were determined for all lines and compared to measurement of the same compounds in transgenic and control lines grown under low light conditions. The results are summarized in Table 1.

Transgenic plants exhibited a higher NADH content under high light than control plants, and produced less reactive oxygen species under high light than control plants. No difference was observed between constructs wherein the encoded NAD salvage pathway enzyme was equipped with a nuclear localization signal or not.

Transgenic plant lines were also phenotypically scored for tolerance to high light stress conditions. To this end, plants were grown in vitro at low light conditions (30 µEinstein m⁻² sec⁻¹) for two weeks and transferred for 3 days to high light conditions (250 µEinstein m⁻² sec⁻¹; 16 hrs light -8hrs dark). After the high light treatment the plants were returned to low light conditions and grown for another three days before scoring the phenotype.

Whereas control plants were small, and had started flowering (stress-induced), the plants of the transgenic lines comprising the chimeric genes as described in Example 1 were larger than the control plants and only had started to bolt.

Table 1. High light tolerance of transgenic *Arabidopsis* lines over-expressing the chimeric yeast genes as described in Example 1.

Chimeric genes	Segregation for PPT tolerance	% NADH versus low light control		% superoxides versus low light control	
		Low light	High light	Low light	High light
Control	-	100	68	100	145
PNC1 (NLS) line 1	3:1	108	128	80	73
PNC1 (NLS) line 2	3:1	139	128	82	76
NPT1 line 1	6:1	128	147	66	70
NPT1 line 2	6:1	122	135	82	76
NPT1 (NLS)	12:1	106	150	61	80

STANDARD ERROR OF MEAN < 10%

Example 3: protocols for measurement of NADH content and superoxide content.

**Intracellular NAD(P)H quantification
using a water-soluble tetrazolium salt**

Reference

Jun Nakamura, Shoji Asakura, Susan D. Hester, Gilbert de Murcia, Keith W. Caldecott and James A. Swenberg (2003) Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single strand break repair in base excision repair deficient cells in real time. *Nucleic Acids Research* 31(17), e104.

Plant material

Most plant material can be used:

- In vitro grown *Arabidopsis* shoots 14-18 days old but NOT flowering
- Hypocotyl explants of oilseed rape

Cell Counting Kit-8 (CCK-8)

Sopachem n.v./Belgium
72A, Avenue du Laarbeeklaan - 1090 Brussels
Belgium

Contents:

5mL bottles containing 5mMol/L WST-8 (tetrazolium salt), 0.2mMol/L 1-Methoxy PMS, 150mMol/L NaCl

Reaction solution:

- 10mL 25mM K-phosphate buffer pH7.4
- 0.5mL CCK-8

- 0.1mM 1-Methoxy-5-methylphenazinium methyl sulfate (= 1-Methoxyphenazine methosulfate): 1µL/mL of 100mM stock (MW= 336.4; 100mg in 2.973mL water)
- 1 drop Tween20/25mL

Procedure

- Harvest plant material and put in 25mM K-phosphate buffer pH7.4
 - e.g.: 150 oilseed rape hypocotyl explants
 - 1gr Arabidopsis shoots (without roots)
- Replace buffer with reaction solution
 - 15mL for 1gr Arabidopsis shoots
 - 15mL for 150 oilseed rape hypocotyl explants
- Incubate at 26°C in the dark for about 1/2 hour (follow reaction)
- Measure the absorbance of the reaction solution at 450nm

Measuring superoxide production by quantifying the reduction of XTT

Ref.: De Block, M., De Brouwer, D. (2002) A simple and robust in vitro assay to quantify the vigour of oilseed rape lines and hybrids. Plant Physiol. Biochem. 40, 845-852

A. BRASSICA NAPUS**Media and reaction buffers**

Sowing medium (medium 201):

Half concentrated Murashige and Skoog salts
2% sucrose
pH 5.8
0.6% agar (Difco Bacto Agar)
250mg/l triacillin

Callus inducing medium A2S3:

MS medium, 0.5g/l Mes (pH 5.8), 3% sucrose, 40mg/l adenine-SO₄,
0.5% agarose, 1mg/l 2,4-D, 0.25mg/l NAA, 1mg/l BAP, 250mg/l
triacillin

Reaction buffer:

25mM K-phosphate buffer pH 8
1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) = XTT (BioVectra, Canada) (MW 674.53)

Dissolve XTT by careful warming solution ($\pm 37^{\circ}\text{C}$) (cool down to room temperature before use)

1 drop Tween20 for 25ml buffer

Sterilization of seeds - pregermination of seeds - growing of the seedlings

Seeds are soaked in 70% ethanol for 2 min, then surface-sterilized for 15 min in a sodium hypochlorite solution (with about 6% active chlorine) containing 0.1% Tween20. Finally, the seeds are rinsed with 1l of sterile tap water.

Incubate seeds for at least one hour in sterile tap water (to allow diffusion from seeds of components that may inhibit germination).

Seeds are put in 250ml erlenmeyer flasks containing 50ml of sterile tap water (+ 250mg/l triacillin). Shake for about 20 hours.

Seeds from which the radicle is protruded are put in Vitro Vent containers from Duchefa containing about 125ml of sowing medium (10 seeds/vessel, not too many to reduce loss of seed by contamination). The seeds are germinated at $\pm 24^{\circ}\text{C}$ and $10\text{-}30\mu\text{Einstein s}^{-1}\text{m}^{-2}$ with a daylength of 16h.

P.S.: For calculating the amount of seeds that have to be sown: 5 hypocotyl segments/seedling

Preculture of the hypocotyl explants and induction of stress

- 12-14 days after sowing, the hypocotyls are cut in about 7-10mm segments.
- The hypocotyl explants (25 hypocotyls/Optilux Petridish, Falcon S1005, Denmark) are cultured for 5 days on medium A2S3 at 25°C (at $10\text{-}30\mu\text{Einstein s}^{-1}\text{m}^{-2}$).

P.S.: 150 hypocotyl explants are used per condition.

- Induction of stress:

Transfer hypocotyl explants to A2S3 medium containing respectively 0, 25 and 50mg/l acetylsalicylic acid.

Incubate for about 24 hours at 25°C and $10\text{-}30\mu\text{Einstein s}^{-1}\text{m}^{-2}$ with a

daylength of 16h.

XTT-assay

- Transfer 150 hypocotyl explants to a 50ml Falcon tube.
- Wash with reaction buffer (without XTT).
- Add 20mL reaction buffer + XTT.
(explants have to be submerged, but do not vacuum infiltrate)
- Incubate in the dark at 26°C
- Follow the reaction by measuring the absorption of the reaction medium at 470nm

B. ARABIDOPSIS THALIANA

Media and reaction buffers

Plant medium:

Half concentrated Murashige and Skoog salts
B5 vitamins
1.5% sucrose
pH 5.8
0.7% Difco agar

Incubation medium:

1/2 concentrated MS-salts
1% sucrose
0.5g/L MES pH 5.8
1 drop Tween20 for 25ml medium

Reaction buffer:

25mM K-phosphate buffer pH 8
1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) = XTT (BioVectra, Canada) (MW 674.53)
Dissolve XTT by careful warming solution (\pm 37°C) (cool down to room temperature before use)
1 drop Tween20 for 25ml buffer

Arabidopsis plants

- *Arabidopsis* lines: control (mother line from which tested lines were derived)
lines to test

- Sterilization of *Arabidopsis* seeds:

2min. 70% ethanol

10 min. bleach (6% active chlorine) + 1drop Tween 20 for 20ml solution
wash 5 times with sterile tap water

P.S.: sterilization is done in 2ml eppendorf tubes

Arabidopsis seeds sink to the bottom of the tube,

allowing removal of the liquids by means of a
1ml pipetman

- Pregermination of seeds:

In 9cm Optilux Petridishes (Falcon) containing 12ml sterile tap water.

Low light overnight to 24 hours.

- Growing of *Arabidopsis* plants

Seeds are sown in Intergrid Tissue Culture disks of Falcon (nr. 3025)
containing $\pm 125\text{ml}$ of plant medium: 1 seed/grid.

Plants are grown at 24°C

$30\mu\text{Einstein s}^{-1}\text{m}^{-2}$

16hours light - 8hours dark

for about 18 days (before bolting)

P.S.: 1g of plant material (shoots without roots)/line/condition are
needed to carry

out the assay. 1g shoots corresponds with 40-60 plants.

Induction of stress

Paraquat

- Harvest *Arabidopsis* shoots (without roots)
- Put 1g shoots in incubation medium (shoots have to be submerged, but do not vacuum infiltrate) containing respectively 0, 5 and $10\mu\text{M}$ paraquat
Incubation medium: $\pm 150\text{ml}$ in Intergrid Tissue Culture disks of Falcon (nr. 3025)

- Incubate at 24°C in the dark for ±24 hours and 30-50 μ Einstein s⁻¹m⁻² with a daylength of 16h.

High light

- Transfer half of the plates to high light (250 μ Einstein s⁻¹m⁻²) and incubate for 4 to 20 hours

XTT-assay

- Harvest shoots (without roots) from agar plates (high light stress) or from liquid incubation medium (paraquat stress) and put them in 50ml Falcon tubes containing reaction buffer (without XTT)
- Replace reaction buffer with buffer containing XTT (15mL/gr)
- Shoots have to be submerged, but do not vacuum infiltrate
- Incubate in the dark at 26°C
- Follow the reaction by measuring the absorption of the reaction medium at 470nm (about one hour)

Example 4: Increased ozone tolerance of *Arabidopsis thaliana* plants over-expressing the yeast nicotineamidase (Pnc1) gene.

The chimeric vector pTVE467 (Example 1) was used for transformation of *A. thaliana* ecotype Columbia. Primary transformants were analyzed by Southern-DNA- and Northern-RNA-blot analysis. One transgenic line was identified to carry a single copy of the Pnc1-transgene construct and to have a high steady state level of transgenic full-length Pnc1-mRNA (20 pg/5 µg total RNA).

6 weeks after germination 100 individual plants each of the single copy transgenic line and of wild-type Columbia as a control, were exposed to ozone in fumigation chambers. During 2 consecutive days the plants were treated for 5h/day with ozone concentrations of 250, 350 and 500 ppb respectively. After

treatment all plants were visually screened for ozone injury manifested as necrotic lesions. The results are summarized in Table 2. At 500 ppb ozone exposure nearly all plants showed necrotic lesions whereas at the 2 lower ozone concentrations a significantly lower percentage of transgenic plants were injured.

In addition, the evolution of the vitality performance index (PI) was determined for all plants of the transgenic line and of the wild-type plants under increasing ozone concentration. PI can be calculated by the formula: PI = (ABS/CS) x (TR/CS) x (ET/CS). (ABS = flux of photons absorbed by the antenna pigments Chl*; CS = cross section; TR = energy trapped by the reaction centre and converted into redox energy; ET = electron flux further downstream leading to CO₂ fixation) In the transgenic line, the vitality performance index PI significantly increased with increasing ozone concentrations whereas this index remains constant in wild-type plants treated with increasing ozone concentrations. This can be explained by a physiological compensation response within the transgenic line to counteract the ozone damage.

Table 2. Increased ozone tolerance of *Arabidopsis thaliana* plants over-expressing the yeast nicotineamidase (Pnc1) gene.

	250 ppb O ₃	350 ppb O ₃	500 ppb O ₃
Wild-type	45%*	50%	100%
Pnc1	20%	25%	100%

* percentage of the plants exhibiting necrotic lesions

Furthermore, control plants, homozygous transgenic populations of plants comprising the chimeric Pnc1 gene as well as a heterozygous transgenic population, were subjected to ozone fumigations and scored for visible injury and various physiological responses compared to non-fumigated plants. The assessment included measurement of non-modulated fluorescence, modulated fluorescence, chlorophyll measurement and fresh weight determination.

Based on the visible injury and physiological responses, a ranking was made for each population indicating the degree of the ozone impact. The more negative the evaluation, the more sensitive the population's response to ozone.

Whereas the control non-transgenic population and the heterozygous transgenic population had a cumulative score of -13, the two homozygous transgenic populations had a score of -6 and -2 respectively. It is therefore clear that the homozygous transgenic populations performed statistically significantly better than the control plants.

Claims

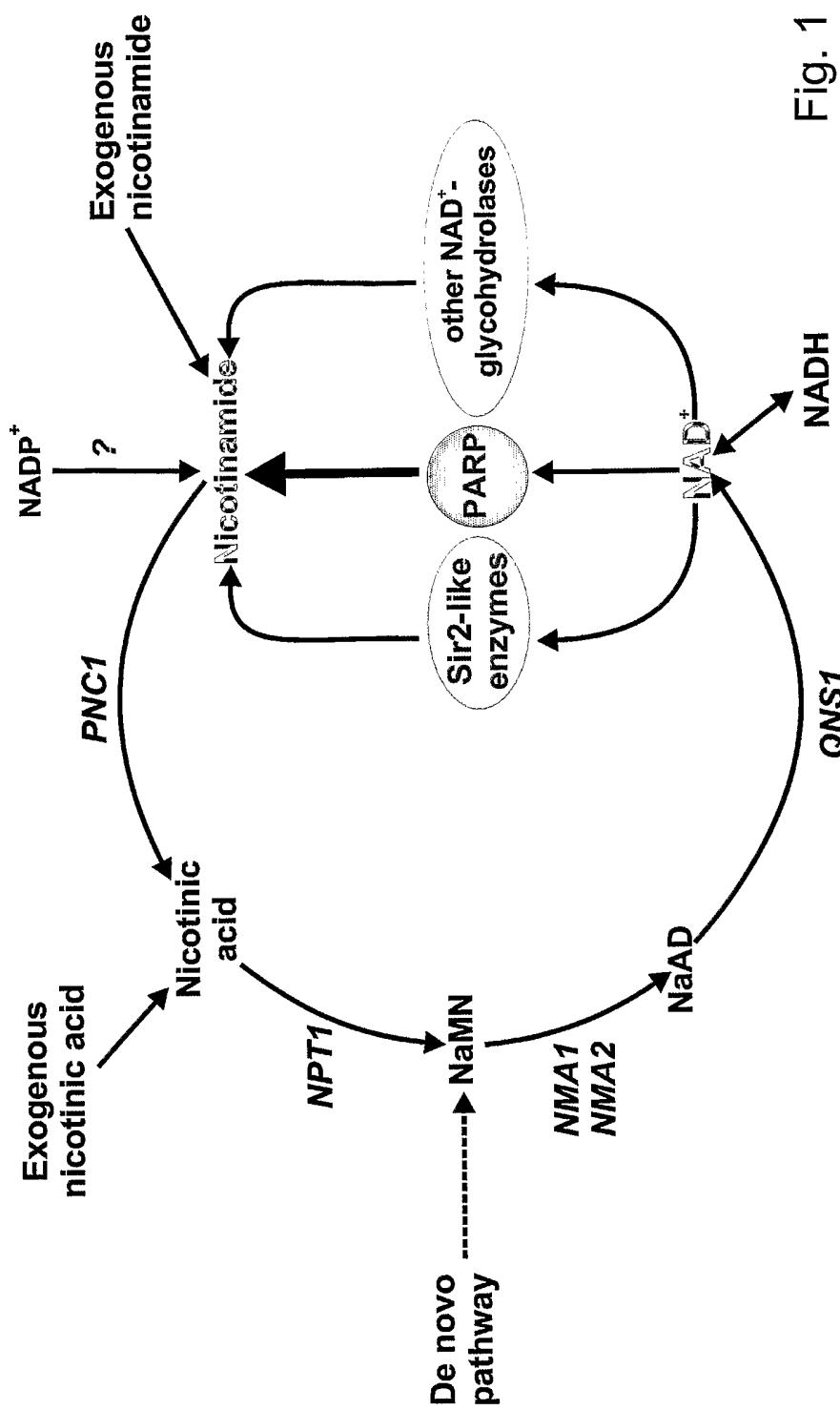
1. A method for obtaining a plant with increased stress resistance comprising
 - a. introducing a chimeric gene into a cells of a plant to obtain transgenic cells, said chimeric gene comprising the following operably linked DNA fragments:
 - i. A plant-expressible promoter;
 - ii. A DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase;
 - iii. A 3'end region involved in transcription termination and polyadenylation;
 - b. regenerating said transgenic cells to obtain a population of transgenic plants; and
 - c. selecting a plant from said population of transgenic plants which exhibits increased stress resistance or selecting a plant which exhibits a reduced level of reactive oxygen species or maintains a high level of NADH under stress conditions when compared to a similar non-transgenic plant.
2. The method according to claim 1, wherein said DNA region codes for an enzyme comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway.

3. The method according to claim 1, wherein said DNA region codes for nicotinamidase.
4. The method according to claim 3, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.:2, SEQ ID No.:12, SEQ ID No.:14 or SEQ ID No.:16.
5. The method according to claim 3, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 1, SEQ ID No.:3, SEQ ID No.:11, SEQ ID No.:13 or SEQ ID No.: 15.
6. The method according to claim 1, wherein said DNA region codes for nicotinate phosphoribosyltransferase.
7. The method according to claim 6, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 4, SEQ ID No.: 18 or SEQ ID No.: 20.
8. The method according to claim 6 wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 17 or SEQ ID No.: 19.
9. The method according to claim 1, wherein said DNA region codes for nicotinic acid mononucleotide adenyltransferase.
10. The method according to claim 9, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 6, SEQ ID No.: 8 or SEQ ID No.: 22.
11. The method according to claim 9, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 5, SEQ ID No.: 7 or SEQ ID No.: 21.

12. The method according to claim 1, wherein said DNA region codes for NAD synthetase.
13. The method according to claim 12, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 10 or SEQ ID No.: 24.
14. The method according to claim 12, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 9 or SEQ ID No.: 23.
15. The method according to any one of claims 1 to 14, further comprising the step of crossing said plant with another plant.
16. A chimeric gene as described in any one of claims 1 to 14.
17. A plant cell comprising a chimeric gene as described in claim 16.
18. A plant comprising a chimeric gene as described in claim 16.
19. The plant of claim 18 which is a plant selected from cotton, *Brassica* vegetables, oilseed rape, wheat, corn or maize, barley, sunflower, rice, oats, sugarcane, soybean, vegetables, chicory, lettuce, tomato, tobacco, potato, sugarbeet, papaya, pineapple, mango or *Arabidopsis thaliana*.
20. The plant according to claim 18 or 19, further characterized in that it has a lower level of reactive oxygen species under stress conditions than a similar plant not comprising such a chimeric gene.
21. A seed of a plant according to claim 18 or claim 19 comprising a chimeric gene according to claim 16.

22. Use of a chimeric gene according to claim 16 to increase the stress resistance of a plant.
23. Use of a chimeric gene according to claim 16 to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.
24. Use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase to increase the stress resistance of a plant.
25. Use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.
26. Use of a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway to increase the stress resistance of a plant.

27. Use of a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.
28. Use of a DNA sequence comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23 to increase the stress resistance of a plant.
29. Use of a DNA sequence comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23 to increase the stress resistance of a plant. to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.



NaMN: nicotinic acid mononucleotide
NaAD: desamino-NAD⁺

PNC1: nicotinamidase
NPT1: nicotinate phosphoribosyl transferase
NMA: NAD⁺ pyrophosphorylase
QNS1: NAD⁺ synthetase

Fig. 2

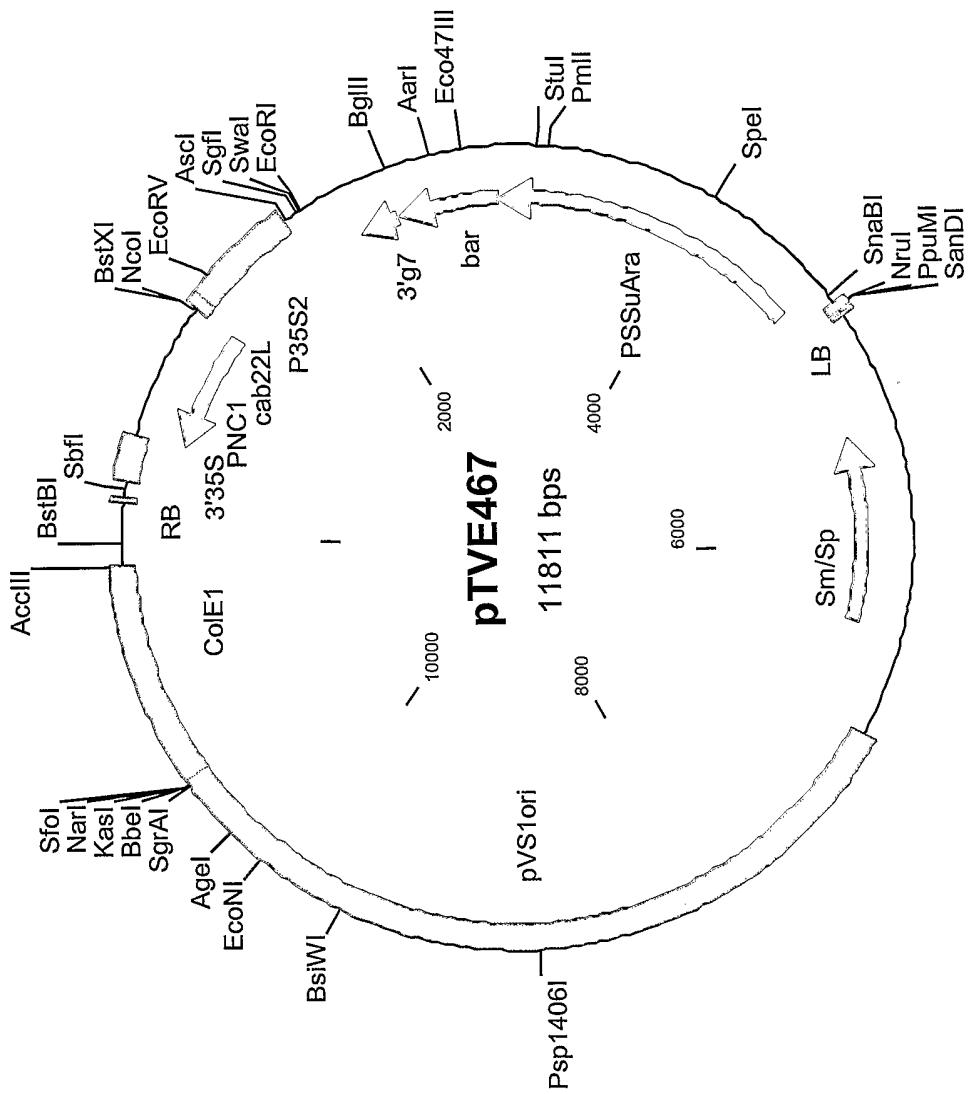


Fig. 3

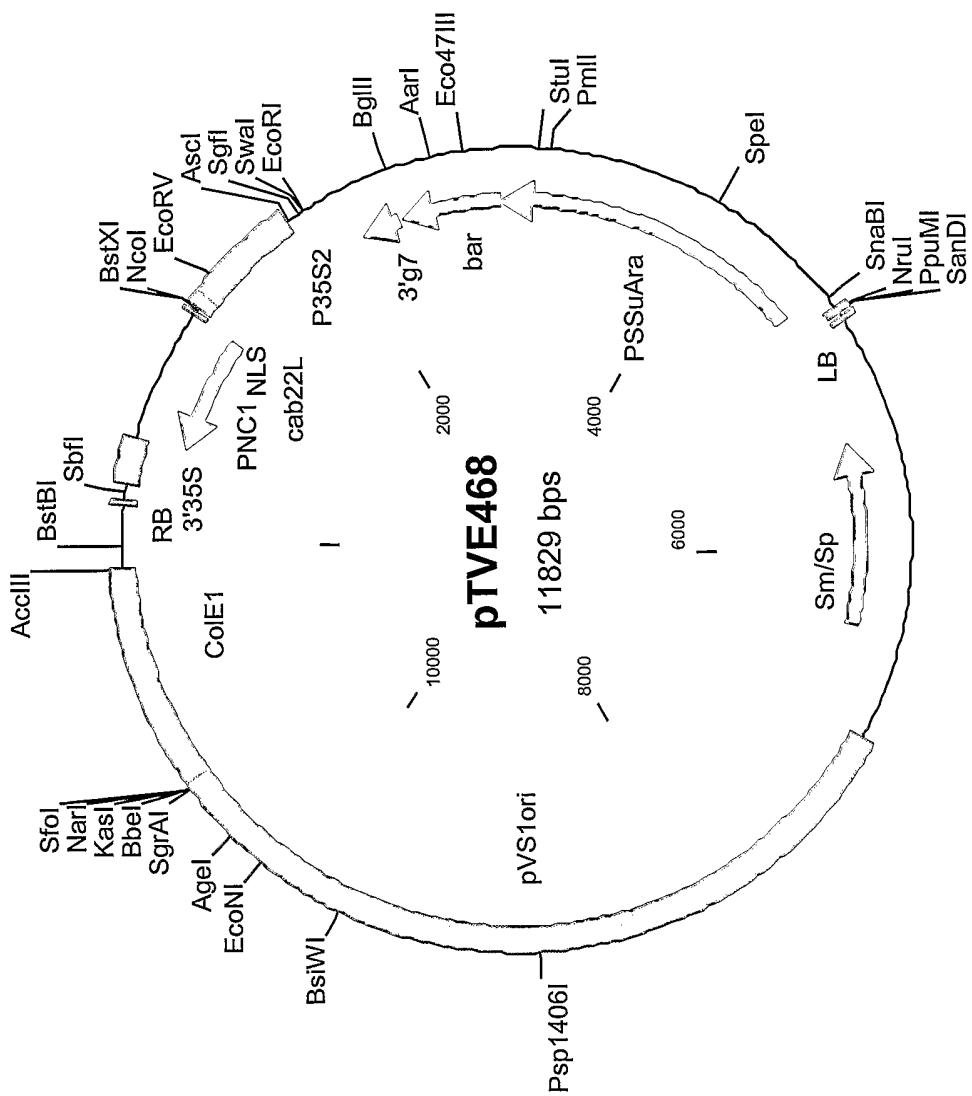


Fig. 4

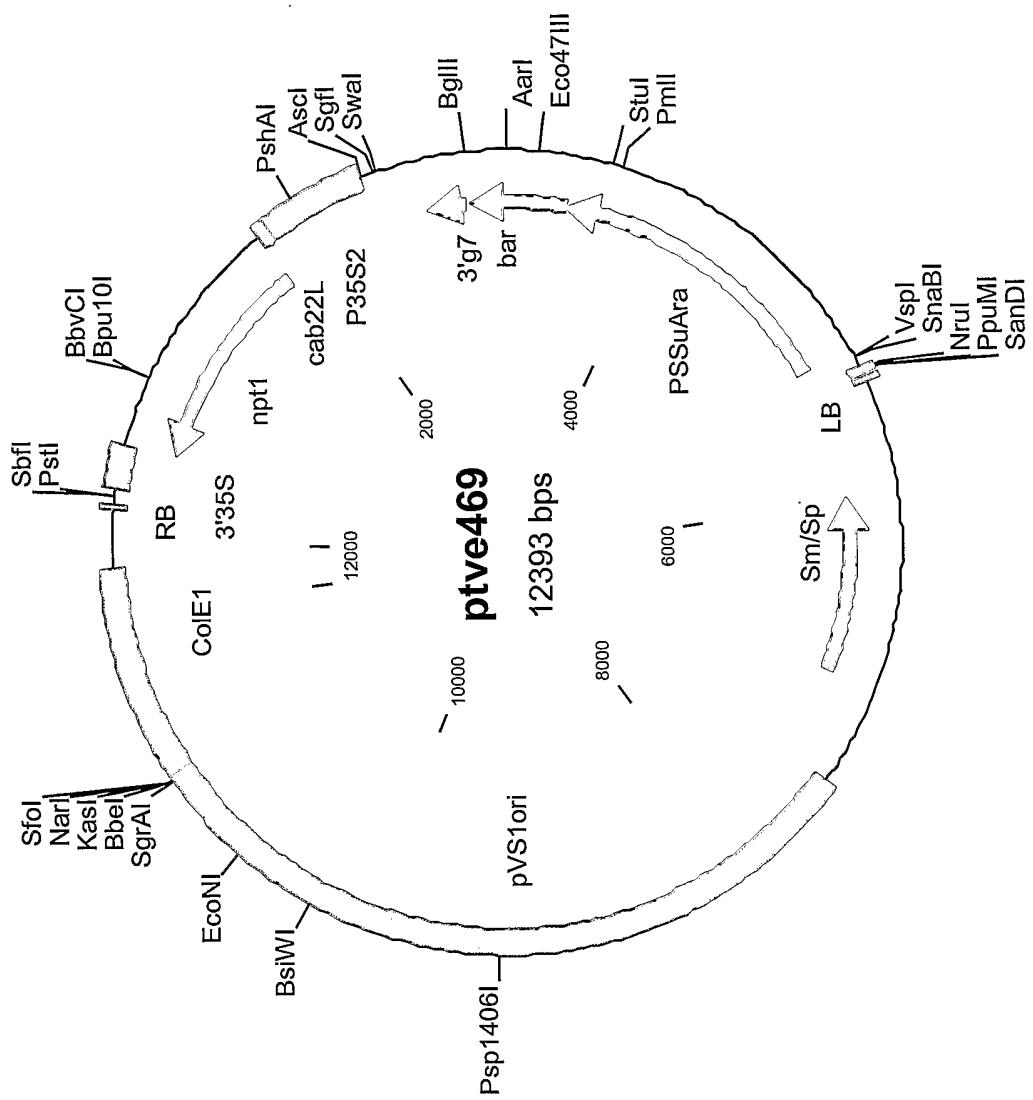


Fig. 5

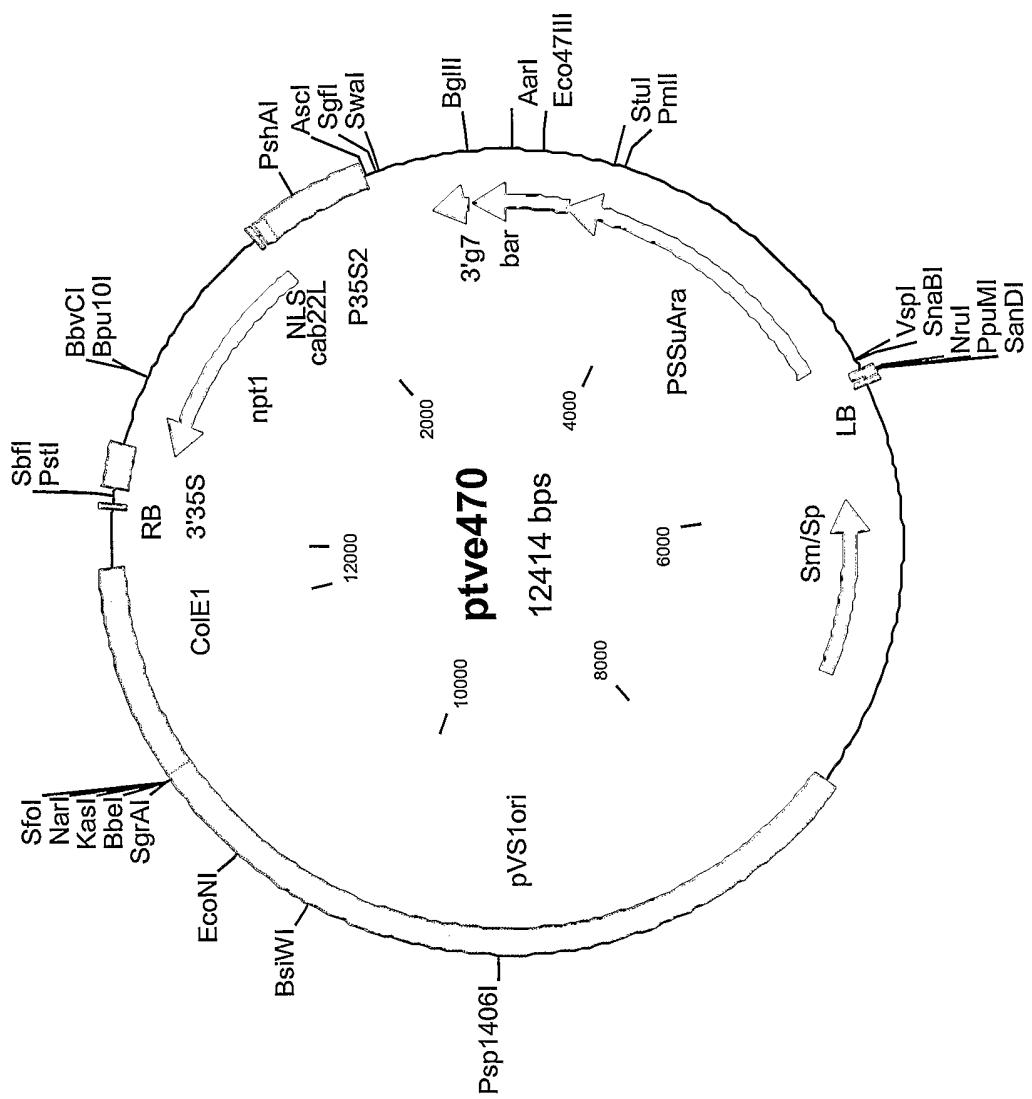


Fig. 6

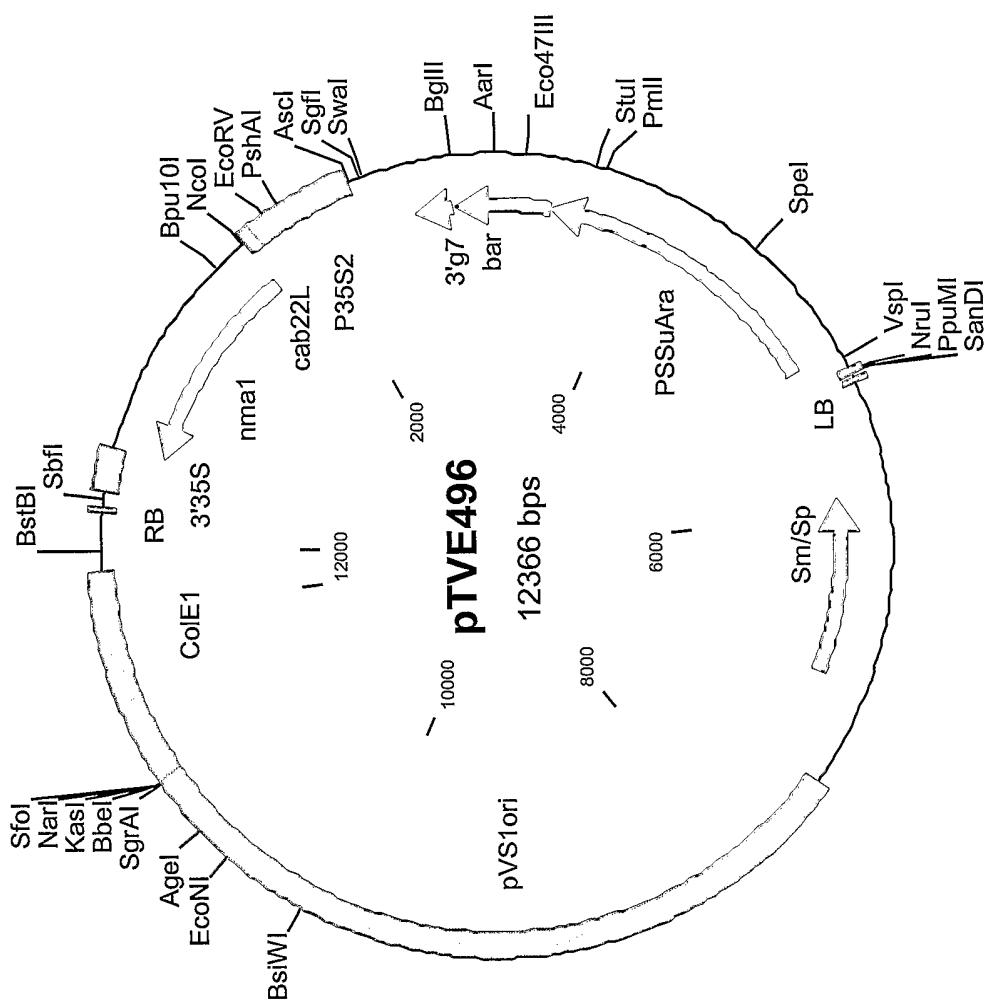


Fig. 7

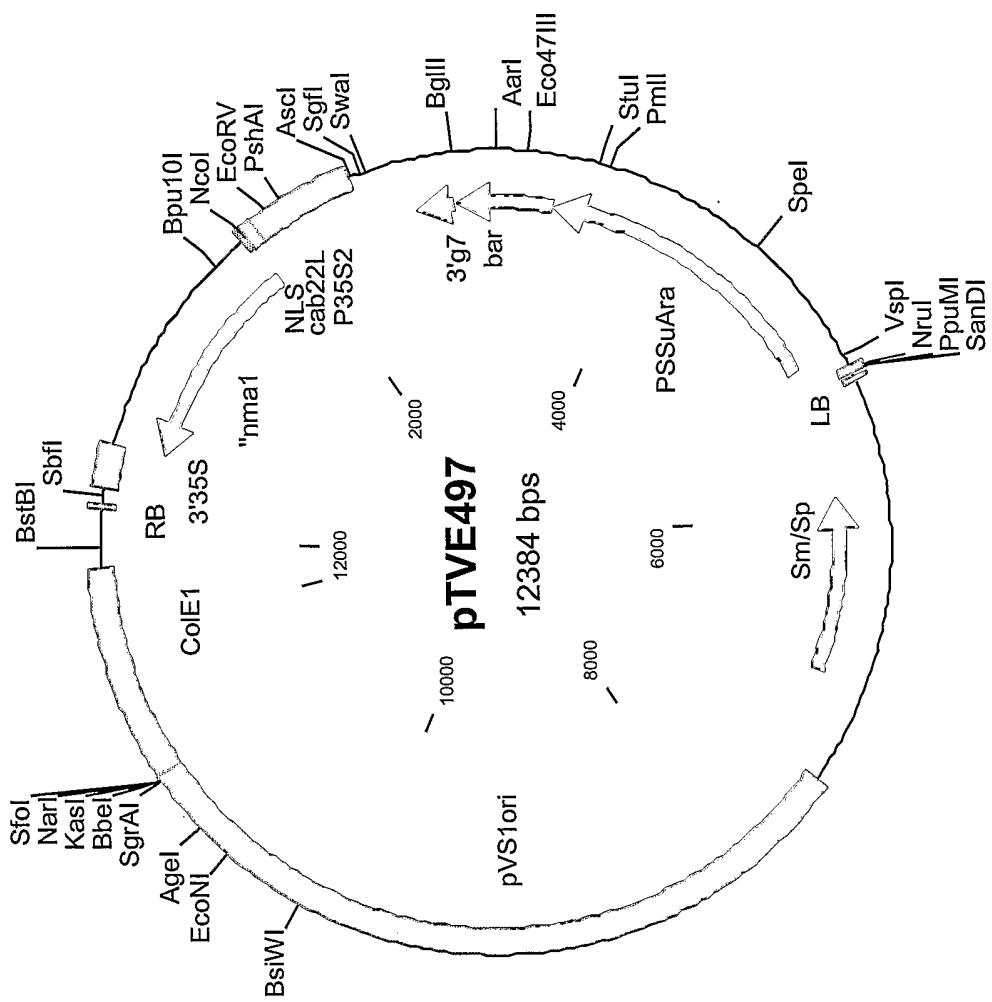


Fig. 8

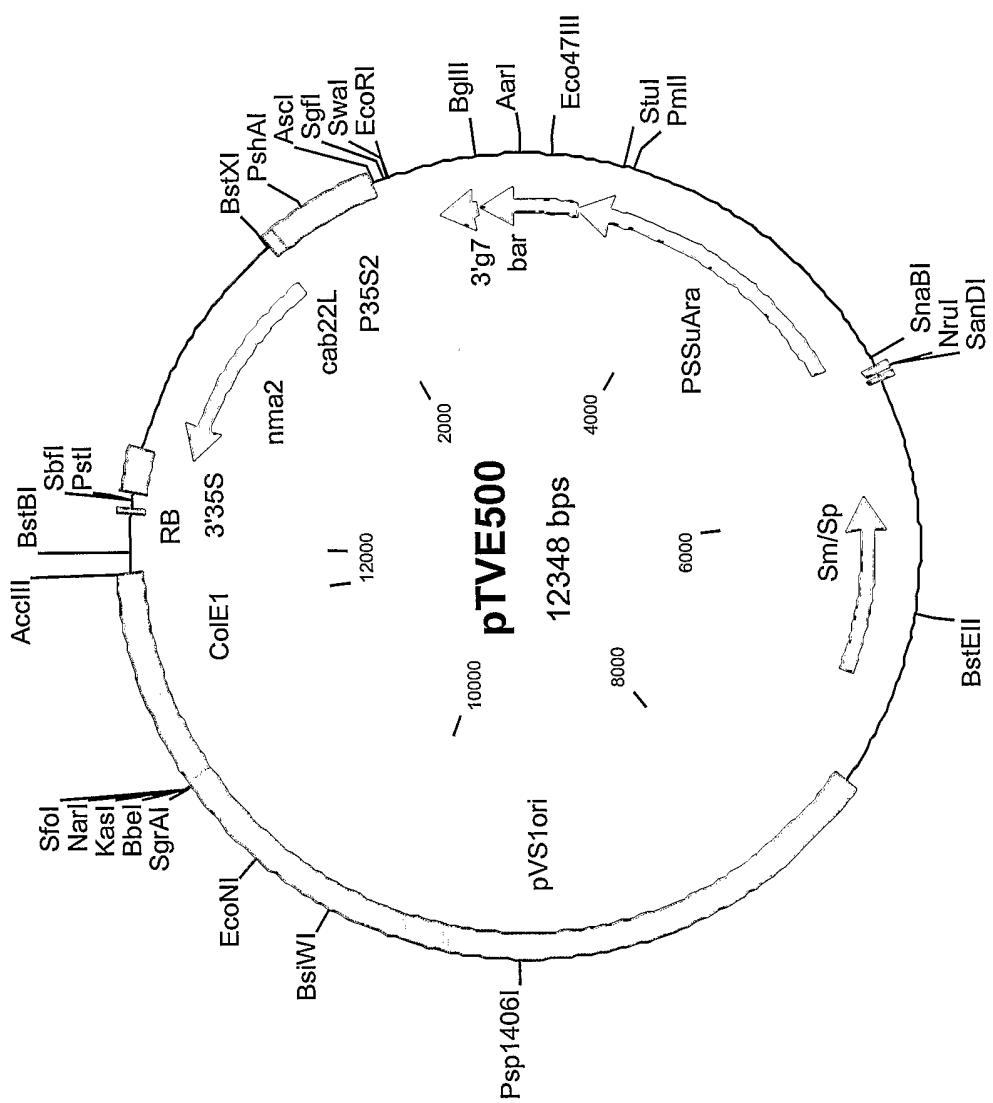


Fig. 9

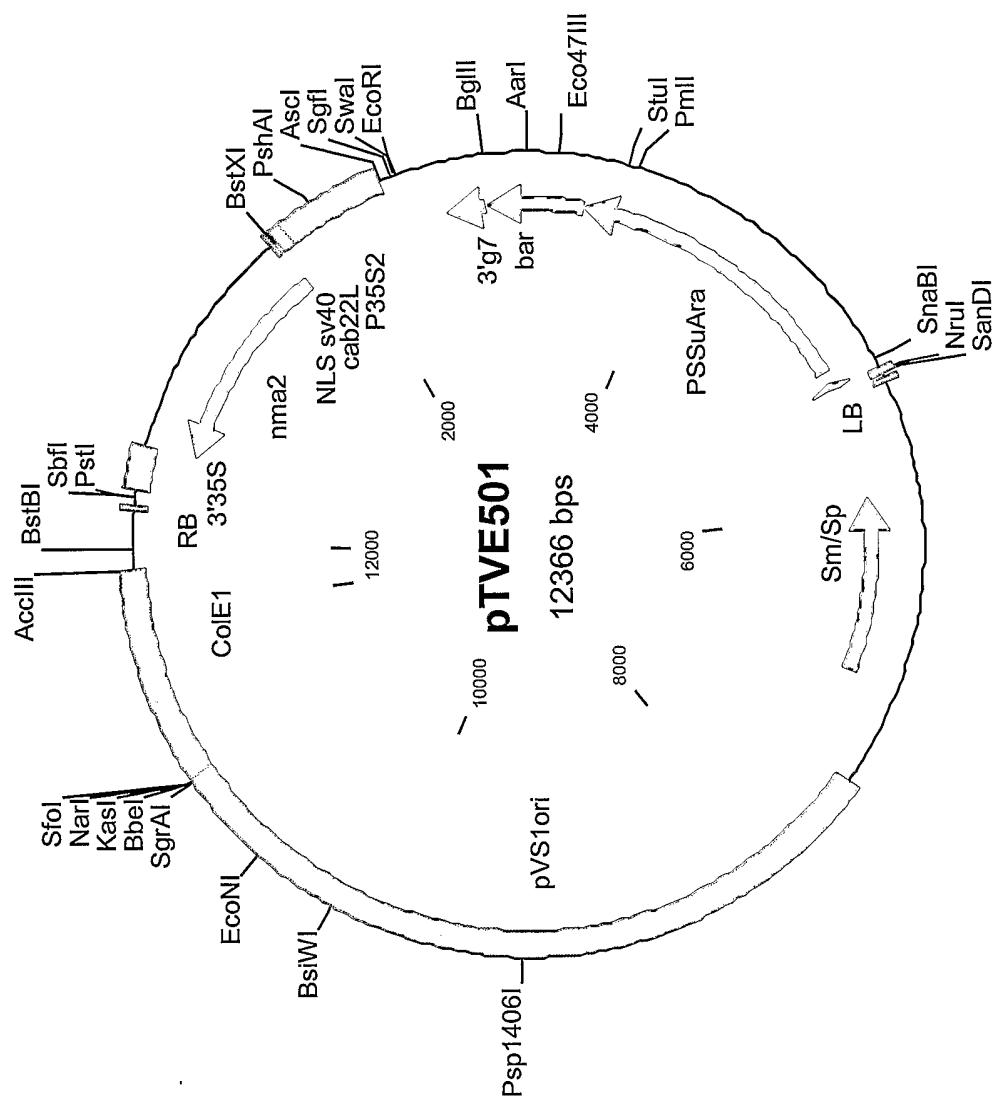


Fig. 10

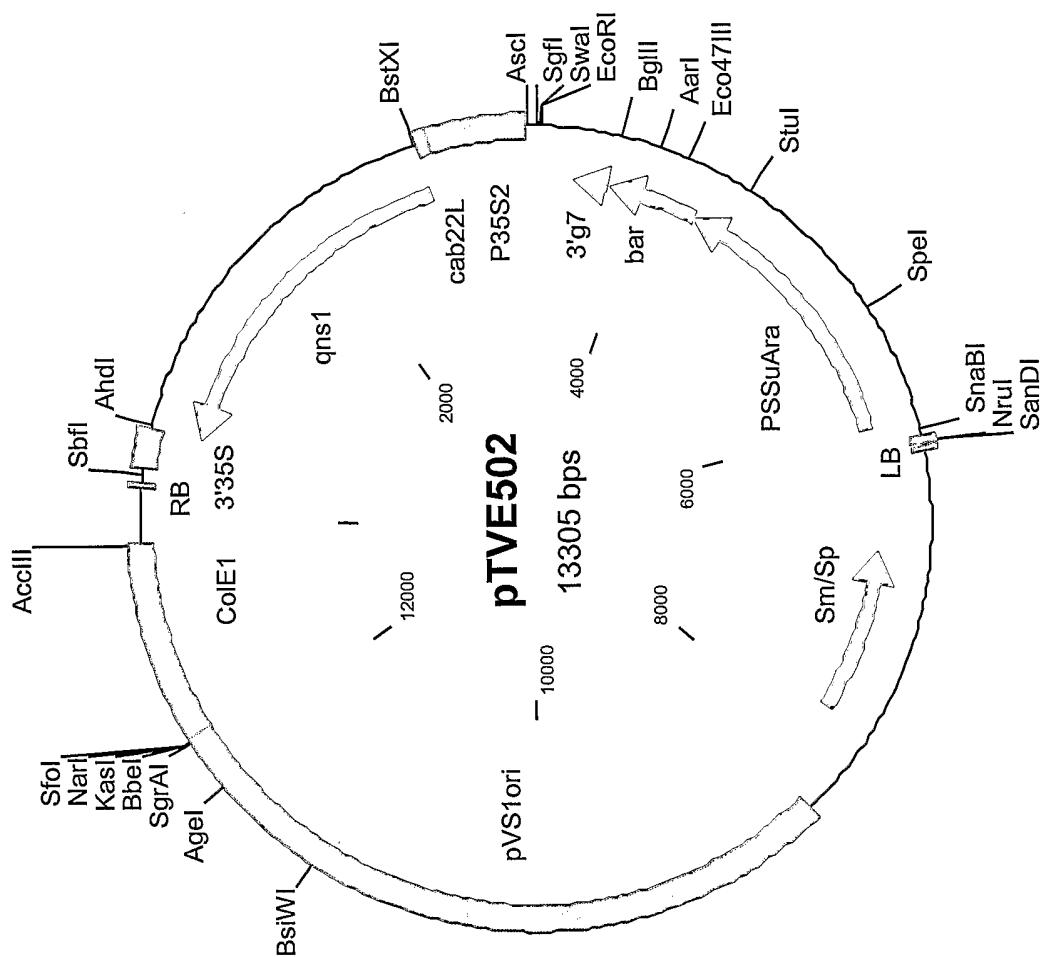
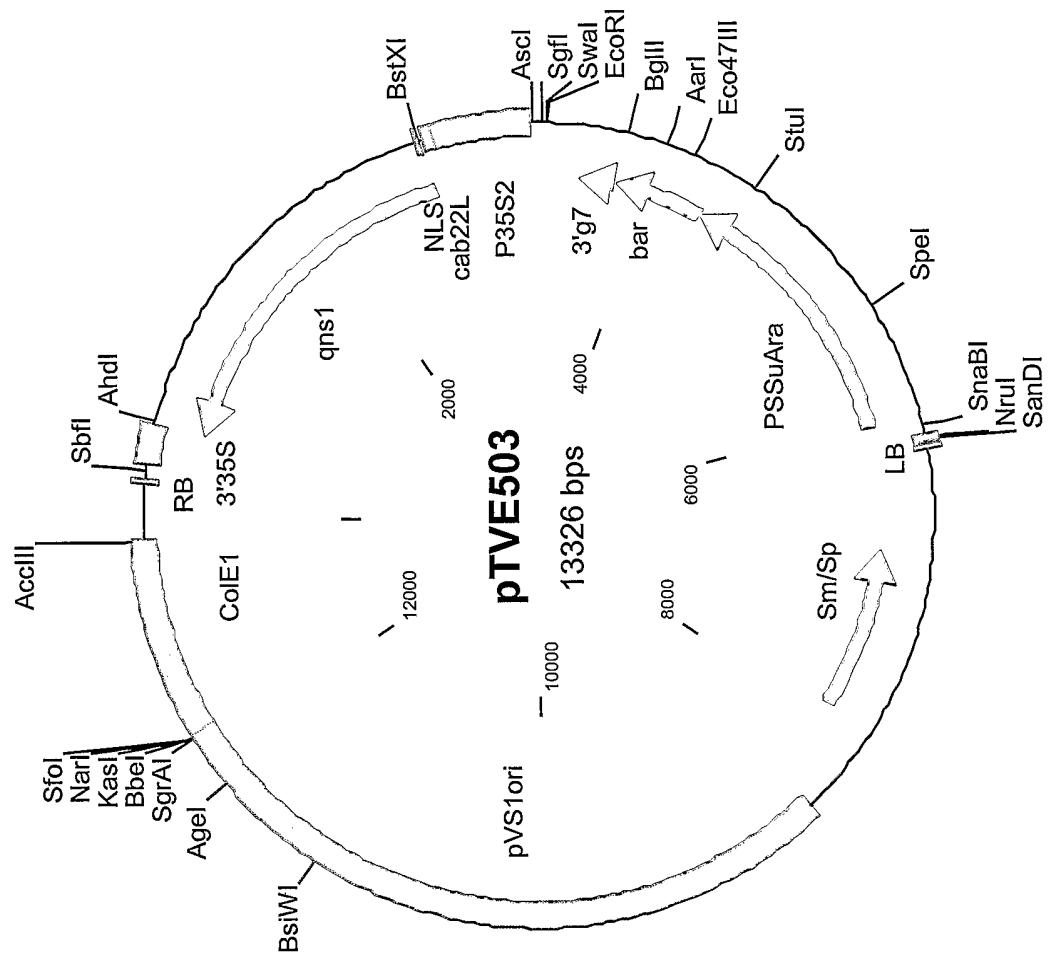


Fig. 11



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Metzlaff, Michael

Gosselé, Veronique

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

Gly Asp Leu Ile Glu Ser Met Gly Glu Pro Asn Val Trp Ala Asp Ala
 290 295 300
 Asp Leu His His Ile Leu Gly Asn Tyr Gly Cys Leu Ile Val Glu Arg
 305 310 315 320
 Thr Gly Ser Asp Val Arg Ser Phe Leu Leu Ser His Asp Ile Met Tyr
 325 330 335
 Glu His Arg Arg Asn Ile Leu Ile Lys Gln Leu Ile Tyr Asn Asp
 340 345 350
 Ile Ser Ser Thr Lys Val Arg Leu Phe Ile Arg Arg Ala Met Ser Val
 355 360 365
 Gln Tyr Leu Leu Pro Asn Ser Val Ile Arg Tyr Ile Gln Glu His Arg
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<210> 7

<211> 1188

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 7

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attgacgcta	ctctacagga	tgttacgatg	actggaaatt	tgggggtact	gaagagccaa	360
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ccagtaagt	ataactatca	aaagcgaggg	ttagccccag	cttatcatcg	tgtccgcattg	660
tgcaattag	catgcgagcg	gacatcatct	tggtaatgg	ttgatgcctg	ggaatcttta	720
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tccttcttgc	tttccatga	tatcatgtat	gaacacagaa	gaaatatcct	tattatcaaa	1020
caacttattt	acaatgatat	ttcctctacg	aaagtgcggc	tttcatcag	acgtggaaatg	1080
tcagttcaat	atcttcttcc	aaactctgtc	atccgttaca	tccaaagagta	taatctatac	1140
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<210> 8

<211> 395

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 8

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					20				25				30		
Pro	Ile	Val	Pro	Tyr	Val	Leu	Ala	Asp	Tyr	Asn	Ser	Ser	Ile	Asp	Ala
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Pro	Phe	Asn	Leu	Asp	Ile	Tyr	Lys	Thr	Leu	Ser	Ser	Arg	Lys	Lys	Asn
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Ala	Asn	Ser	Ser	Asn	Arg	Met	Asp	His	Ile	Pro	Leu	Asn	Thr	Ser	Asp
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Phe	Gln	Pro	Leu	Ser	Arg	Asp	Val	Ser	Ser	Glu	Glu	Glu	Ser	Glu	Gly
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Gln	Ser	Asn	Gly	Ile	Asp	Ala	Thr	Leu	Gln	Asp	Val	Thr	Met	Thr	Gly
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Asn	Leu	Gly	Val	Leu	Lys	Ser	Gln	Ile	Ala	Asp	Leu	Glu	Glu	Val	Pro
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Val	His	Arg	Leu	Thr	Lys	Lys	Leu	Gln	Asp	Pro	Glu	Lys	Leu	Pro	Leu
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Ile	Ile	Val	Ala	Cys	Gly	Ser	Phe	Ser	Pro	Ile	Thr	Tyr	Leu	His	Leu
					165				170			175			
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Arg	Gly	Leu	Ala	Pro	Ala	Tyr	His	Arg	Val	Arg	Met	Cys	Glu	Leu	Ala
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Cys	Glu	Arg	Thr	Ser	Ser	Trp	Leu	Met	Val	Asp	Ala	Trp	Glu	Ser	Leu
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Gln	Ser	Ser	Tyr	Thr	Arg	Thr	Ala	Lys	Val	Leu	Asp	His	Phe	Asn	His
					245				250			255			
Glu	Ile	Asn	Ile	Lys	Arg	Gly	Gly	Ile	Met	Thr	Val	Asp	Gly	Glu	Lys
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Met	Gly	Val	Lys	Ile	Met	Leu	Leu	Ala	Gly	Gly	Asp	Leu	Ile	Glu	Ser
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Gly	Asn	Tyr	Gly	Cys	Leu	Ile	Val	Glu	Arg	Thr	Gly	Ser	Asp	Val	Arg
					305				310			315			320
Ser	Phe	Leu	Leu	Ser	His	Asp	Ile	Met	Tyr	Glu	His	Arg	Arg	Asn	Ile
					325				330			335			
Leu	Ile	Ile	Lys	Gln	Leu	Ile	Tyr	Asn	Asp	Ile	Ser	Ser	Thr	Lys	Val
					340				345			350			
Arg	Leu	Phe	Ile	Arg	Arg	Gly	Met	Ser	Val	Gln	Tyr	Leu	Leu	Pro	Asn
					355				360			365			
Ser	Val	Ile	Arg	Tyr	Ile	Gln	Glu	Tyr	Asn	Leu	Tyr	Ile	Asn	Gln	Ser
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<210> 9

<211> 2145

<212> DNA

<213> *Saccharomyces cerevisiae*

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cgtgtcgcc cagaactgga aataaactggc tacggatgtt tagatcattt ttttagaaaat	180	
gacgtttgcc ttcatcattt gaaaaatgtat gctcaaataa ttaagaataa agaaaaccat	240	
ggattaatac ttgacatttg tatgcccgtt ctacacaaga atgttcgtt taattgtcgt	300	
tttgttatccc tggatggta gatatttttc ataagaccta agatttgggtt agctaattgtat	360	
ggtaactata gggaaatgag atttttcaca ccttggatga aacctggcgt ggtggaggac	420	
tttattccctt cacctgagat tcagaaagtt accggccaga gacttgcgtt atttggggac	480	
gctgtgataa attcatttgg tacatgcatt ggtacagaaa cttgtgaaga attgttaca	540	
cctcaatcccc cccacatcgc catgtcttta gatgggtgtgg aaatcatgac aaactcatct	600	
ggttctcatc atgaactgcg taagttaaat aaaaggttag acctaattttt aaatgcact	660	
aaacgttgtt gtgggttta ctgttatgca aatcaaagag gttgtgtatgg tgacagat	720	
tattatgtatg gctgtgcact aattgtccatc aatggtacaa ttgttagccca aggttcacaa	780	
tttgcgttag atgatgttgg agtagttact gctactgtgg acctagaaga ggtgaggagt	840	
tatcgtgcag ctgtcatgtc tcgtggccta caagccctt tggcagaaat aaagtcaag	900	
cgtattgata ttccctgtaga attggcttta atgaccccca gatttgcgtt tacagtgtgt	960	
ccaaacaaaag tccgcgagcc tttctatcac tctcctgagg aagaaattgc actggacac	1020	
gcttgcttggta tggggattttt ttaagacgt tgtaacggaa cagggtttt ccttccctta	1080	
tctggggca ttgactcttgc tgcaactgca atgattgtcc actctatgtg ccgttttagtg	1140	
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agccgcgtatg attggattcc agacagtcca caggatcttag cctcaaaaat atttcactcc	1260	
tgtttcatgg gtacggaaaa ttcatccaag gagacaagaa acagagcaaa ggaccttcc	1320	
aatgcaattt gatcttacca cgtggattta aagatggact cattggatc cagtgtgg	1380	
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ggtagcgcaa atgttgcgtt gtcgttacgt gggtatctaa caaaatatga ctgctcctcc	1620	
gcagatatac accctattgg ggttattca aaaactgact tggaaaagatt cattgcctac	1680	
gcatcaaaaac aatataaacat gccaatcttgc aatgacttt taaacgtac accaactgca	1740	
gaattagaac ctatgactaa agattacgtt caatcgtatc agatagat gggatgacg	1800	
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atgttctttaa aacttcttca tcaatggtcc ccaaaggtaa cacctcgta aatatctgaa	1920	
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cctagttatc atgctgaaca gtattcacca gaagacaaca gatttgactt acgtccccc	2040	
ttaatcaacc caagatttcc atgggcttca agaaaaatttgc atgaagttgt cgagcgtgt	2100	
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<210> 10

<211> 714

<212> PRT

<213> *Saccharomyces cerevisiae*

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 Ala Lys Glu Arg Gly Ala Arg Leu Arg Val Gly Pro Glu Leu Glu Ile
 35 40 45
 Thr Gly Tyr Gly Cys Leu Asp His Phe Leu Glu Asn Asp Val Cys Leu
 50 55 60
 His Ser Trp Glu Met Tyr Ala Gln Ile Ile Lys Asn Lys Glu Thr His
 65 70 75 80
 Gly Leu Ile Leu Asp Ile Gly Met Pro Val Leu His Lys Asn Val Arg
 85 90 95
 Tyr Asn Cys Arg Leu Leu Ser Leu Asp Gly Glu Ile Leu Phe Ile Arg
 100 105 110
 Pro Lys Ile Trp Leu Ala Asn Asp Gly Asn Tyr Arg Glu Met Arg Phe
 115 120 125
 Phe Thr Pro Trp Met Lys Pro Gly Val Val Glu Asp Phe Ile Leu Pro
 130 135 140
 Pro Glu Ile Gln Lys Val Thr Gly Gln Arg Leu Val Pro Phe Gly Asp
 145 150 155 160
 Ala Val Ile Asn Ser Leu Asp Thr Cys Ile Gly Thr Glu Thr Cys Glu
 165 170 175
 Glu Leu Phe Thr Pro Gln Ser Pro His Ile Ala Met Ser Leu Asp Gly
 180 185 190
 Val Glu Ile Met Thr Asn Ser Ser Gly Ser His His Glu Leu Arg Lys
 195 200 205
 Leu Asn Lys Arg Leu Asp Leu Ile Leu Asn Ala Thr Lys Arg Cys Gly
 210 215 220
 Gly Val Tyr Leu Tyr Ala Asn Gln Arg Gly Cys Asp Gly Asp Arg Leu
 225 230 235 240
 Tyr Tyr Asp Gly Cys Ala Leu Ile Ala Ile Asn Gly Thr Ile Val Ala
 245 250 255
 Gln Gly Ser Gln Phe Ser Leu Asp Asp Val Glu Val Val Thr Ala Thr
 260 265 270
 Val Asp Leu Glu Glu Val Arg Ser Tyr Arg Ala Ala Val Met Ser Arg
 275 280 285
 Gly Leu Gln Ala Ser Leu Ala Glu Ile Lys Phe Lys Arg Ile Asp Ile
 290 295 300
 Pro Val Glu Leu Ala Leu Met Thr Ser Arg Phe Asp Pro Thr Val Cys
 305 310 315 320
 Pro Thr Lys Val Arg Glu Pro Phe Tyr His Ser Pro Glu Glu Ile
 325 330 335
 Ala Leu Gly Pro Ala Cys Trp Met Trp Asp Tyr Leu Arg Arg Cys Asn
 340 345 350
 Gly Thr Gly Phe Phe Leu Pro Leu Ser Gly Gly Ile Asp Ser Cys Ala
 355 360 365
 Thr Ala Met Ile Val His Ser Met Cys Arg Leu Val Thr Asp Ala Ala
 370 375 380
 Gln Asn Gly Asn Glu Gln Val Ile Lys Asp Val Arg Lys Ile Thr Arg
 385 390 395 400
 Ser Gly Asp Asp Trp Ile Pro Asp Ser Pro Gln Asp Leu Ala Ser Lys
 405 410 415
 Ile Phe His Ser Cys Phe Met Gly Thr Glu Asn Ser Ser Lys Glu Thr
 420 425 430

Arg Asn Arg Ala Lys Asp Leu Ser Asn Ala Ile Gly Ser Tyr His Val
 435 440 445
 Asp Leu Lys Met Asp Ser Leu Val Ser Ser Val Val Ser Leu Phe Glu
 450 455 460
 Val Ala Thr Gly Lys Lys Pro Ile Tyr Lys Ile Phe Gly Gly Ser Gln
 465 470 475 480
 Ile Glu Asn Leu Ala Leu Gln Asn Ile Gln Ala Arg Leu Arg Met Val
 485 490 495
 Leu Ser Tyr Leu Phe Ala Gln Leu Leu Pro Trp Val Arg Gly Ile Pro
 500 505 510
 Asn Ser Gly Gly Leu Leu Val Leu Gly Ser Ala Asn Val Asp Glu Cys
 515 520 525
 Leu Arg Gly Tyr Leu Thr Lys Tyr Asp Cys Ser Ser Ala Asp Ile Asn
 530 535 540
 Pro Ile Gly Gly Ile Ser Lys Thr Asp Leu Lys Arg Phe Ile Ala Tyr
 545 550 555 560
 Ala Ser Lys Gln Tyr Asn Met Pro Ile Leu Asn Asp Phe Leu Asn Ala
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 Thr Pro Thr Ala Glu Leu Glu Pro Met Thr Lys Asp Tyr Val Gln Ser
 580 585 590
 Asp Glu Ile Asp Met Gly Met Thr Tyr Glu Glu Leu Gly Val Phe Gly
 595 600 605
 Tyr Leu Arg Lys Val Glu Lys Cys Gly Pro Tyr Ser Met Phe Leu Lys
 610 615 620
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 625 630 635 640
 Lys Val Lys Arg Phe Phe Phe Tyr Ala Ile Asn Arg His Lys Gln
 645 650 655
 Thr Val Leu Thr Pro Ser Tyr His Ala Glu Gln Tyr Ser Pro Glu Asp
 660 665 670
 Asn Arg Phe Asp Leu Arg Pro Phe Leu Ile Asn Pro Arg Phe Pro Trp
 675 680 685
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<210> 11

<211> 597

<212> DNA

<213> *Arabidopsis thaliana*

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aacaacgttc	tcaccaccat	cgacatctgc	cgacgcgcct	cagtccccgt	attcttacg	180
cgtcacaacc	acaaatcccc	gaccgaccac	ggcatgctcg	gcgagtggtg	taacggcgat	240
gtaatccctt	acggaaccac	cgattctgaa	atcatccagg	agatacaagg	ccaagtaacc	300
ggaccagacg	agatggtgga	gaagaacacg	tacagtgcgt	ttaacaaaac	ccgcctccag	360
gaaaacctgg	aaaagatcgg	agtaaaggag	gtgatcgtga	tcggagtgtat	gacgaacttg	420
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gacgcgactg	cgacgtttaa	tgaggagctt	cacgaggcta	cgctaattgaa	tctcgctttt	540

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<211> 198

<212> PRT

<213> *Arabidopsis thaliana*

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Ser Ser Met Ala Lys Pro Ile Leu Asn Asn Val Leu Thr Thr Ile Asp	35	40	45	
Ile Cys Arg Arg Ala Ser Val Pro Val Phe Phe Thr Arg His Asn His	50	55	60	
Lys Ser Pro Thr Asp His Gly Met Leu Gly Glu Trp Cys Asn Gly Asp	65	70	75	80
Val Ile Leu Asp Gly Thr Thr Asp Ser Glu Ile Ile Gln Glu Ile Gln	85	90	95	
Gly Gln Val Thr Gly Pro Asp Glu Met Val Glu Lys Asn Thr Tyr Ser	100	105	110	
Ala Phe Asn Lys Thr Arg Leu Gln Glu Asn Leu Glu Lys Ile Gly Val	115	120	125	
Lys Glu Val Ile Val Ile Gly Val Met Thr Asn Leu Cys Cys Glu Thr	130	135	140	
Thr Ala Arg Glu Ala Phe Ile Lys Gly Phe Arg Val Phe Phe Ser Thr	145	150	155	160
Asp Ala Thr Ala Thr Phe Asn Glu Glu Leu His Glu Ala Thr Leu Met	165	170	175	
Asn Leu Ala Phe Gly Phe Ala Tyr Leu Val Asp Cys Asp Lys Leu Arg	180	185	190	
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<210> 13

<211> 597

<212> DNA

<213> *Arabidopsis thaliana*

<400> 13

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caaaacgctc tcaccacat cgacatctgc cgacgcgtt caatccccgt attcttacg	180
cggcacaacc acaaatcccc aaccgaccac ggcatgtcg gagagtggtg gaacggcgat	240
ctaattcctcg acggaaccac tgattccgaa atcatcccgg aaatcaatcg ccaggtcacc	300
ggaccagacg aaatcgatcgaa gaagagcaccg tacagtggtt ttaacaacac gcaccttcag	360
gagaagctgg acaagatcggtt agtgaaggag gtgatcgat tcggagtgtt gacgaacctaa	420

tgctgtgaga cgacggcgcg tgaagcgttt gtaaaagggtt ttagggtttt tttctcgacg	480
gacgcgactg cgacggtaa tgaagagctt cacgaggcta ctctaattgaa tctcgcgtat	540
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<210> 14

<211> 198

<212> PRT

<213> Arabidopsis thaliana

<400> 14

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Tyr Ser Met Ala Glu Pro Ile Leu Gln Asn Ala Leu Thr Thr Ile Asp	
35 40 45	
Ile Cys Arg Arg Ala Ser Ile Pro Val Phe Phe Thr Arg His Asn His	
50 55 60	
Lys Ser Pro Thr Asp His Gly Met Leu Gly Glu Trp Trp Asn Gly Asp	
65 70 75 80	
Leu Ile Leu Asp Gly Thr Thr Asp Ser Glu Ile Ile Pro Glu Ile Asn	
85 90 95	
Arg Gln Val Thr Gly Pro Asp Glu Ile Val Glu Lys Ser Thr Tyr Ser	
100 105 110	
Ala Phe Asn Asn Thr His Leu Gln Glu Lys Leu Asp Lys Ile Gly Val	
115 120 125	
Lys Glu Val Ile Val Ile Gly Val Met Thr Asn Leu Cys Cys Glu Thr	
130 135 140	
Thr Ala Arg Glu Ala Phe Val Lys Gly Phe Arg Val Phe Phe Ser Thr	
145 150 155 160	
Asp Ala Thr Ala Thr Val Asn Glu Glu Leu His Glu Ala Thr Leu Met	
165 170 175	
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Arg Gly Leu Leu Ser Ser	
195	

<210> 15 .

<211> 591

<212> DNA

<213> Arabidopsis thaliana

<400> 15

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cgtcaaggaa gagatgttga attattcagg cggccataact acagttctga gaaagtccgg	240
ccagttatta aaggcacccgt aggagcagaa ttgggttgatg gattgtatgat caacgaagaa	300

gatgactata agattgtgaa aactcgttc agtgcttct ttagtaccaa tcttcattcc	360
ttcttgcaaa cttcaggggt taccaagtta gtgattgctg gtgtgaaac gccgaactgt	420
atccggcaaa cggtgttga tgcagtggcg ctggattatc ccaatgtgac tgttattaca	480
gatgccacag ctgctgcaac accagagatc catactgcga atattctga catgaagaat	540
attggagtca agactcctac attacacgag tggtccgaag aacttgctt a	591

<210> 16

<211> 196

<212> PRT

<213> Arabidopsis thaliana

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Ser Ile Val Pro Asn Val Ile Arg Val Val Glu Leu Ala Arg Gln Arg	
35 40 45	
Gly Ile Leu Val Ile Trp Val Val Arg Glu His Asp Arg Gln Gly Arg	
50 55 60	
Asp Val Glu Leu Phe Arg Arg His Asn Tyr Ser Ser Glu Lys Val Gly	
65 70 75 80	
Pro Val Ile Lys Gly Thr Val Gly Ala Glu Leu Val Asp Gly Leu Met	
85 90 95	
Ile Asn Glu Glu Asp Asp Tyr Lys Ile Val Lys Thr Arg Phe Ser Ala	
100 105 110	
Phe Phe Ser Thr Asn Leu His Ser Phe Leu Gln Thr Ser Gly Val Thr	
115 120 125	
Lys Leu Val Ile Ala Gly Val Gln Thr Pro Asn Cys Ile Arg Gln Thr	
130 135 140	
Val Phe Asp Ala Val Ala Leu Asp Tyr Pro Asn Val Thr Val Ile Thr	
145 150 155 160	
Asp Ala Thr Ala Ala Ala Thr Pro Glu Ile His Thr Ala Asn Ile Leu	
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Asp Met Lys Asn Ile Gly Val Lys Thr Pro Thr Leu His Glu Trp Ser	
180 185 190	
Glu Glu Leu Ala	
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<210> 17

<211> 1680

<212> DNA

<213> Arabidopsis thaliana

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tattggaaag	ctggcaaaca	atctgagcga	tctgtgtttg	atctgtattt	tcgtaagaat	180
cctttggtg	gagaatacac	tatcttgct	ggtttagaaag	aatgcataa	atttctcgct	240
aatttcaatt	tgactgtat	agagatcgat	ttcggtcg	attcggtacc	tggatgttag	300
gaagcttct	gtgattatct	tcgaggcctt	gattgtctg	acattgaagt	gtatgccatt	360
tcgaaaggat	cagttgttt	tcctaaagtt	cctttactca	gaatcgaagg	tcctgttgct	420
gtgggtcaat	tgttggaaac	tccattcctc	aatctcatca	attacgcattc	tttggttgct	480
acaaatgcag	caagacatcg	gtttgtgc	ggaaaatcta	agttctgtct	tgagtttggt	540
gctagaagag	ctcaggacc	cgatggtc	ataagcgcatt	caaagtattt	ctaccttgg	600
ggttttgatg	caacaagtaa	tgttgcagcg	ggaaaactgt	ttggatacc	cctccgtggt	660
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gagcttgcag	cgttcatc	atatgcactt	gcattccaa	actcctttct	cgctcttgta	900
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aatgaatttg	gatacaaagc	agtaggcatt	agactggatt	cagtgactt	agcctatctt	1020
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caagctgcgt	taggttgtt	tttcaaactt	gtggaaataa	acaatcagcc	tcggatcaaa	1260
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gaagagctag	agccattgaa	agagctaaga	aatcgttgc	tcaaacagct	cgaaaatatg	1560
cgaccggatc	atatgagaag	attaaaccct	actcctata	aggttagt	cagcgccaag	1620
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<210> 18

<211> 559

<212> PRT

<213> *Arabidopsis thaliana*

<400> 18

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

145	150	155	160
Thr Asn Ala Ala Arg His Arg Phe Val Ala Gly Lys Ser Lys Leu Leu			
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Leu Glu Phe Gly Ala Arg Arg Ala Gln Gly Pro Asp Gly Ala Ile Ser			
180	185	190	
Ala Ser Lys Tyr Cys Tyr Leu Gly Gly Phe Asp Ala Thr Ser Asn Val			
195	200	205	
Ala Ala Gly Lys Leu Phe Gly Ile Pro Leu Arg Gly Thr His Ser His			
210	215	220	
Ala Phe Val Ser Ser Phe Met Ser Leu Asp Glu Ile Val Asp Lys Val			
225	230	235	240
Leu Arg Ser Ser Asp Gly Lys Ser Thr Cys Lys Asp Phe Ile Cys Leu			
245	250	255	
Val Gln Thr Cys Leu Thr Lys Ile Gln Asn Ser Ser Leu Gln Gly			
260	265	270	
Ile Phe Ser Glu Thr Asn Gln Ser Glu Leu Ala Ala Phe Ile Ser Tyr			
275	280	285	
Ala Leu Ala Phe Pro Asn Ser Phe Leu Ala Leu Val Asp Thr Tyr Asp			
290	295	300	
Val Met Lys Ser Gly Ile Pro Asn Phe Cys Ala Val Ala Leu Ala Leu			
305	310	315	320
Asn Glu Leu Gly Tyr Lys Ala Val Gly Ile Arg Leu Asp Ser Gly Asp			
325	330	335	
Leu Ala Tyr Leu Ser Thr Glu Val Arg Lys Phe Phe Cys Ala Ile Glu			
340	345	350	
Arg Asp Leu Lys Val Pro Asp Phe Gly Lys Met Ile Val Thr Ala Ser			
355	360	365	
Asn Asp Leu Asn Glu Glu Thr Val Asp Ala Leu Asn Lys Gln Gly His			
370	375	380	
Glu Val Asp Ala Phe Gly Ile Gly Thr Asn Leu Val Thr Cys Tyr Ala			
385	390	395	400
Gln Ala Ala Leu Gly Cys Val Phe Lys Leu Val Glu Ile Asn Asn Gln			
405	410	415	
Pro Arg Ile Lys Leu Ser Glu Asp Val Thr Lys Val Ser Ile Pro Cys			
420	425	430	
Lys Lys Arg Thr Tyr Arg Leu Phe Gly Lys Glu Gly Tyr Pro Leu Val			
435	440	445	
Asp Ile Met Thr Gly Glu Asn Glu Pro Pro Pro Lys Val Gly Glu Arg			
450	455	460	
Leu Leu Cys Arg His Pro Phe Asn Glu Ser Lys Arg Ala Tyr Val Val			
465	470	475	480
Pro Gln Arg Val Glu Glu Leu Leu Lys Cys Tyr Trp Arg Gly Asn Ala			
485	490	495	
Asp Glu Ala Arg Glu Glu Leu Glu Pro Leu Lys Glu Leu Arg Asn Arg			
500	505	510	
Cys Ile Lys Gln Leu Glu Asn Met Arg Pro Asp His Met Arg Arg Leu			
515	520	525	
Asn Pro Thr Pro Tyr Lys Val Ser Val Ser Ala Lys Leu Tyr Asp Phe			
530	535	540	
Ile His Phe Leu Trp Leu Asn Glu Ala Pro Val Gly Glu Leu His			
545	550	555	

<210> 19

<211> 1674

<212> DNA

<213> Arabidopsis thaliana

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aaagctggca aacacaaoa acgatccgtt ttcgatctgt attttcgtaa gaacccattt	180
ggtggtgagt acactgtgt tgctggatta gaagagtgtg ttaagttctt agccaatttc	240
aaattgactg atgaagaaaat cgatttcgtt caagagtgtt tgctctggatc tgaggaagct	300
tttgtgatt atcttagagg gcttgattgt tctgatgtt aagtttatgc aattccggaa	360
ggatcagttg ttttcctaa agtaccttc atgagagtgg aaggaccgtt tgggtttgtt	420
caattgttg aaactccatt cctcaatott gtcaattttg catctttgggt agctactaac	480
gcagctaggc atcgctttgt tgccggaaaa tctaagagtc tactcgagtt tggtgctcga	540
agggctcagg gtccggatgg tgcataataagc gcatcaaaaat attgctaccc tggagggttt	600
gatgcaacaa gtaatgttagc agctggaaaa ctttttggga ttcctcttcg tggAACACAC	660
tctcatgctt atgttagctc attcatgagt actgatgaga ttgttgacaa agtacttcgt	720
agtgcgtatg ggaaaaccac gtgcgaggat ttgttagtc atgttcagac atggtaaaaa	780
aagattcagt attcaccatc tctaagtggc attttctctg agacaaatca aagcgagcta	840
gcagctttca cctcatatgc actggcatc cccaaaactt ttcttgcctt cgtagataca	900
tacatgtga tgaagagtgg aatcccataac ttctgtgcag ttgttttagc actcaatgac	960
tttggatata aagcatttagg tattagactg gattcaggtg atttagctt totatctaga	1020
gaggccagaa atttcttctg cacggtagag agagaactaa aagtgcctgg ttttgggaag	1080
atgtcgtca ctgctagtaa tgatctaaat gaagagacga ttgacgcctt aaataaacag	1140
ggacatgagg tggatgcctt tggcatcggg acctacttgg tcacttgcta ttccacaagcg	1200
gccttaggtt gcgttttcaa acttgtggag ataaacaatc agcctcgat taaaactttct	1260
gaagatgtta caaaggatc aataccgtgt aaaaagcgaa gttacagatt atacggcaa	1320
gaaggttacc ctctggtaga tataatgact ggagagaacg aaccacccctt aaagggttgtt	1380
gagcgtttac ttgtcgta cccattcaac gaatccaaaaa gagcatatgt agtgcaccaa	1440
cgtgtcgaag agctcctcaa atgttattgg cgtgaaagtg cagatgaagc aagagaagta	1500
ttaccgcctt taaaaagat aagagaccgt tgcatcaaac agctcgaaaa catgcacact	1560
gatcatatga ggagattaaa cccaaactcct tataaggta gtgtaaagcgc aaagctgtac	1620
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<210> 20

<211> 557

<212> PRT

<213> Arabidopsis thaliana

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Phe Thr Met Ala Tyr Ala Tyr Trp Lys Ala Gly Lys His Asn Glu Arg	
35 40 45	
Ser Val Phe Asp Leu Tyr Phe Arg Lys Asn Pro Phe Gly Gly Glu Tyr	
50 55 60	
Thr Val Phe Ala Gly Leu Glu Glu Cys Val Lys Phe Leu Ala Asn Phe	
65 70 75 80	

Lys Leu Thr Asp Glu Glu Ile Asp Phe Val Gln Glu Cys Leu Pro Gly
 85 90 95
 Ser Glu Glu Ala Phe Cys Asp Tyr Leu Arg Gly Leu Asp Cys Ser Asp
 100 105 110
 Val Glu Val Tyr Ala Ile Pro Glu Gly Ser Val Val Phe Pro Lys Val
 115 120 125
 Pro Leu Met Arg Val Glu Gly Pro Val Gly Val Val Gln Leu Leu Glu
 130 135 140
 Thr Pro Phe Leu Asn Leu Val Asn Phe Ala Ser Leu Val Ala Thr Asn
 145 150 155 160
 Ala Ala Arg His Arg Phe Val Ala Gly Lys Ser Lys Ser Leu Leu Glu
 165 170 175
 Phe Gly Ala Arg Arg Ala Gln Gly Pro Asp Gly Ala Ile Ser Ala Ser
 180 185 190
 Lys Tyr Cys Tyr Leu Gly Gly Phe Asp Ala Thr Ser Asn Val Ala Ala
 195 200 205
 Gly Lys Leu Phe Gly Ile Pro Leu Arg Gly Thr His Ser His Ala Tyr
 210 215 220
 Val Ser Ser Phe Met Ser Thr Asp Glu Ile Val Asp Lys Val Leu Arg
 225 230 235 240
 Ser Ala Asp Gly Lys Thr Thr Cys Glu Asp Phe Val Ser His Val Gln
 245 250 255
 Thr Trp Leu Lys Lys Ile Gln Tyr Ser Pro Ser Leu Ser Gly Ile Phe
 260 265 270
 Ser Glu Thr Asn Gln Ser Glu Leu Ala Ala Phe Thr Ser Tyr Ala Leu
 275 280 285
 Ala Phe Pro Lys Thr Phe Leu Ala Leu Val Asp Thr Tyr Asp Val Met
 290 295 300
 Lys Ser Gly Ile Pro Asn Phe Cys Ala Val Ala Leu Ala Leu Asn Asp
 305 310 315 320
 Phe Gly Tyr Lys Ala Leu Gly Ile Arg Leu Asp Ser Gly Asp Leu Ala
 325 330 335
 Tyr Leu Ser Arg Glu Ala Arg Asn Phe Phe Cys Thr Val Glu Arg Glu
 340 345 350
 Leu Lys Val Pro Gly Phe Gly Lys Met Val Val Thr Ala Ser Asn Asp
 355 360 365
 Leu Asn Glu Glu Thr Ile Asp Ala Leu Asn Lys Gln Gly His Glu Val
 370 375 380
 Asp Ala Phe Gly Ile Gly Thr Tyr Leu Val Thr Cys Tyr Ser Gln Ala
 385 390 395 400
 Ala Leu Gly Cys Val Phe Lys Leu Val Glu Ile Asn Asn Gln Pro Arg
 405 410 415
 Ile Lys Leu Ser Glu Asp Val Thr Lys Val Ser Ile Pro Cys Lys Lys
 420 425 430
 Arg Ser Tyr Arg Leu Tyr Gly Lys Glu Gly Tyr Pro Leu Val Asp Ile
 435 440 445
 Met Thr Gly Glu Asn Glu Pro Pro Pro Lys Val Gly Glu Arg Leu Leu
 450 455 460
 Cys Arg His Pro Phe Asn Glu Ser Lys Arg Ala Tyr Val Val Pro Gln
 465 470 475 480
 Arg Val Glu Glu Leu Leu Lys Cys Tyr Trp Arg Gly Ser Ala Asp Glu
 485 490 495
 Ala Arg Glu Val Leu Pro Pro Leu Lys Glu Ile Arg Asp Arg Cys Ile
 500 505 510
 Lys Gln Leu Glu Asn Met Arg Pro Asp His Met Arg Arg Leu Asn Pro
 515 520 525
 Thr Pro Tyr Lys Val Ser Val Ser Ala Lys Leu Tyr Asp Phe Ile His

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<211> 717

<212> DNA

<213> Arabidopsis thaliana

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tttgagctgg cgagagatga attacgcctca aaaggatttc atgttcttgg aggatatatg	240
tctccctgtta atgatgcata taagaagaag ggcctttat ctgcagaaca tcgttttagag	300
atgtgtaatg tatcatgtca aagctctgac tttgtaatgg ttgatccgtg ggaggcatct	360
caaagcaact accaacgaac tttgacgggtt ttatcaaggg tcaagacttt cttacaacaaca	420
aatcgacatg tacccgagga atctctcaaa gtcatgtac tatgtggctc ggatttactg	480
ctatcttct gcactcccg tggatc cctgaacagt taagaactat ttgcaaagat	540
tatggcattt tggtcatccg tagagaagga caagatgtt aaaaatatgat ctctggtgac	600
gaaatcttaa acgaaaactg tgctaacgtc aaaatcggtt acaatactgt tcctaatacaa	660
atcagttcga gtagattaaag gcaatgcatt tcgcgagggtt ttcgggttaa atacttgact	717
gaagatggag taatagatta tatcagacaa catcaactat acactgagct cacatga	

<210> 22

<211> 238

<212> PRT

<213> Arabidopsis thaliana

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

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

<210> 23

<211> 2178

<212> DNA

<213> Arabidopsis thaliana

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cttggaccgg agctcgaggt cactggctat ggttgcgagg atcacttctt ggaactcgac	180	
actgtcactc atgcgtggaa gtgtttaaag gaatttgatgc ttgtgtattt gacggatgtat	240	
attttgtcga gcataggaat gcctgtgatt aaaggagcag agcgttataa ctgccagggtt	300	
ctctgtatga acagaagaat catcatgatt cgaccggaaa tggcttcgc aaacgatgga	360	
aactataggg agctacgggt gttcacagct tggaaagcaga gagaagagct agaggaattt	420	
cagctccccca ttgaaatttc agaggctttt gggcggaaat cagtcctttt tggttatgtt	480	
tacatccagt ttatcgacac ggctgttgc gctgaagtct gtgaggaact gtttagtcca	540	
cttccttcctc atgcccggct cgcattgaat ggttggtaag tatttatgaa tgcaagtgaa	600	
agtcatcacc aacttaggaa actagatatt cgtctgaatg cttttatggg ggctactcat	660	
gctctgggtt ggggttatat gtacagtaat caacaaggat gcgatggtag ccgcttatac	720	
tcacatggat gtgcattgtat tggtaaac gggaatgtt gttgtcaagg ctcacaattc	780	
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gctgtgccct gtagacttac acagtccttc aacctggaaa tgacactaaag cagtccgaag	960	
aagatcattt accactctcc acaagaagaa atagcctttt gtcccgcttg ctggatgtgg	1020	
gactatttga gaagaagtgg cgcttcaggaa tttttgccttc ctctttctgg cggagcagac	1080	
agctcctccg tggcagctat tggctggctgc atgtgcacac ttgttggtaa agagattgca	1140	
aaggagatg agcaagtaaa agctgatgcg aaccgaattt ggaattatgc taatggcag	1200	
tttcctactg atagcaaaga gtttgcacaa cgaatattttt acactgtctt tatgggttct	1260	
gaaaacagtt ctgaggagac aaaaaggcgt tcaaagcagc tggcagacga gattgggtct	1320	
tggcatcttg atgtttgcattt agatgggtt gttcttcgc ttttatttatttcaaaaca	1380	
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ccttgggttc atagcaaacc aggcttttac cttgttcttag gcagctccaa cgttgcgttac	1560	
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ggaaggatga ggaagatatt ccgttggaa ccagtatcta tggtaaagaa tctatgttac	1860	
aagtggggaa caaagctaag cccagcagaa gtagctgaga aagtgaagta ttcttcaaa	1920	
tattattcga tcaatcgaca caaatgact gtcctcacac cgttcttatca cgctgagat	1980	

tactccccag aggacaacag attcgatctg aggcagttc tgtacaacag caagtggcca 2040
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<210> 24

<211> 725

<212> PRT

<213> Arabidopsis thaliana

<400> 24
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Lys Ala Ala Gly Ala Val Ile Arg Leu Gly Pro Glu Leu Glu Val Thr
35 40 45
Gly Tyr Gly Cys Glu Asp His Phe Leu Glu Leu Asp Thr Val Thr His
50 55 60
Ala Trp Glu Cys Leu Lys Glu Leu Leu Gly Asp Trp Thr Asp Asp
65 70 75 80
Ile Leu Cys Ser Ile Gly Met Pro Val Ile Lys Gly Ala Glu Arg Tyr
85 90 95
Asn Cys Gln Val Leu Cys Met Asn Arg Arg Ile Ile Met Ile Arg Pro
100 105 110
Lys Met Trp Leu Ala Asn Asp Gly Asn Tyr Arg Glu Leu Arg Trp Phe
115 120 125
Thr Ala Trp Lys Gln Arg Glu Glu Leu Glu Phe Gln Leu Pro Ile
130 135 140
Glu Ile Ser Glu Ala Leu Glu Gln Lys Ser Val Pro Phe Gly Tyr Gly
145 150 155 160
Tyr Ile Gln Phe Ile Asp Thr Ala Val Ala Ala Glu Val Cys Glu Glu
165 170 175
Leu Phe Ser Pro Leu Pro Pro His Ala Glu Leu Ala Leu Asn Gly Val
180 185 190
Glu Val Phe Met Asn Ala Ser Gly Ser His His Gln Leu Arg Lys Leu
195 200 205
Asp Ile Arg Leu Asn Ala Phe Met Gly Ala Thr His Ala Arg Gly Gly
210 215 220
Val Tyr Met Tyr Ser Asn Gln Gln Gly Cys Asp Gly Ser Arg Leu Tyr
225 230 235 240
Tyr Asp Gly Cys Ala Cys Ile Val Val Asn Gly Asn Val Val Ala Gln
245 250 255
Gly Ser Gln Phe Ser Leu Arg Asp Val Glu Val Ile Ile Ser Gln Val
260 265 270
Asp Leu Asp Ala Val Ala Ser Leu Arg Gly Ser Ile Ser Ser Phe Gln
275 280 285
Glu Gln Ala Ser Cys Lys Val Lys Val Ser Ser Val Ala Val Pro Cys
290 295 300
Arg Leu Thr Gln Ser Phe Asn Leu Lys Met Thr Leu Ser Ser Pro Lys
305 310 315 320
Lys Ile Ile Tyr His Ser Pro Gln Glu Glu Ile Ala Phe Gly Pro Ala

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Leu	Pro	Leu	Ser	Gly	Gly	Ala	Asp	Ser	Ser	Ser	Val	Ala	Ala	Ile	Val
		355				360								365	
Gly	Cys	Met	Cys	Gln	Leu	Val	Val	Lys	Glu	Ile	Ala	Lys	Gly	Asp	Glu
		370			375									380	
Gln	Val	Lys	Ala	Asp	Ala	Asn	Arg	Ile	Gly	Asn	Tyr	Ala	Asn	Gly	Gln
	385		390				395							400	
Phe	Pro	Thr	Asp	Ser	Lys	Glu	Phe	Ala	Lys	Arg	Ile	Phe	Tyr	Thr	Val
		405				410								415	
Phe	Met	Gly	Ser	Glu	Asn	Ser	Ser	Glu	Glu	Thr	Lys	Arg	Arg	Ser	Lys
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Gln	Leu	Ala	Asp	Glu	Ile	Gly	Ala	Trp	His	Leu	Asp	Val	Cys	Ile	Asp
		435			440									445	
Gly	Val	Val	Ser	Ala	Val	Leu	Ser	Leu	Phe	Gln	Thr	Val	Thr	Gly	Lys
		450			455									460	
Arg	Pro	Arg	Tyr	Lys	Val	Asp	Gly	Gly	Ser	Asn	Ala	Glu	Asn	Leu	Gly
	465		470				475							480	
Leu	Gln	Asn	Ile	Gln	Ala	Arg	Met	Arg	Met	Val	Leu	Ala	Phe	Met	Leu
		485				490								495	
Ala	Ser	Leu	Leu	Pro	Trp	Val	His	Ser	Lys	Pro	Gly	Phe	Tyr	Leu	Val
		500				505								510	
Leu	Gly	Ser	Ser	Asn	Val	Asp	Glu	Gly	Leu	Arg	Gly	Tyr	Leu	Thr	Lys
		515			520									525	
Tyr	Asp	Cys	Ser	Ser	Ala	Asp	Ile	Asn	Pro	Ile	Gly	Ser	Ile	Ser	Lys
		530			535									540	
Met	Asp	Leu	Arg	Leu	Phe	Leu	Lys	Trp	Ala	Ala	Thr	Asn	Leu	Gly	Tyr
	545		550				555							560	
Pro	Ser	Leu	Ala	Glu	Ile	Glu	Ala	Ala	Pro	Pro	Thr	Ala	Glu	Leu	Glu
		565			570									575	
Pro	Ile	Arg	Ser	Asp	Tyr	Ser	Gln	Leu	Asp	Glu	Val	Asp	Met	Gly	Met
		580			585									590	
Thr	Tyr	Glu	Glu	Leu	Ser	Val	Tyr	Gly	Arg	Met	Arg	Lys	Ile	Phe	Arg
	595		600				605								
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