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(54) Title: METHOD FOR PRODUCING A TRANSGENIC PLANT CELL, A PLANT OR A PART THEREOF WITH INCREASED RESISTANCE BIOTIC STRESS

(57) Abstract: The invention relates to the control of pathogens. Disclosed herein are methods of producing transgenic plants with increased pathogen resistance, expression vectors comprising polynucleotides encoding for functional proteins, and transgenic plants and seeds generated thereof.



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Method for producing a transgenic plant cell, a plant or a part thereof with increased resistance biotic stress

[0001.1.1.1] The invention relates to the control of pathogens. Disclosed herein are methods of producing transgenic plants with increased pathogen resistance, expression vectors comprising polynucleotides encoding for functional proteins, and transgenic plants and seeds generated thereof.

[0002.1.1.1] In particular, this invention relates to a transgenic plant cell, a plant or a part thereof with increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control, by increasing or generating one or more activities of biotic stress related protein (BSRP) and to a method for producing said plant cell, a plant or a part thereof.

[0003.1.1.1] The invention also deals with methods of producing and screening for and breeding such plant cells or plants.

[0004.1.1.1] Population increases and climate change have brought the possibility of global food, feed, and fuel shortages into sharp focus in recent years. Additionally, plant performance in terms of growth, development, biomass accumulation and yield depends under field conditions on acclimation ability to the environmental changes and tolerance to plant diseases.

Stresses, biotic and abiotic, exert a critical influence on crop yields. Pathogen attacks are sometimes the most devastating biotic stresses. The enhancement of disease resistance in crops can contribute significantly to increasing the productivity of crops and decreasing the application of pesticides, which can adversely affect human health and the environment when applied in excess.

Plant diseases are infectious diseases which are caused by biotic stress and noninfectious diseases caused by abiotic stress.

Biotic stress is caused by phytopathogens respective bacteria, fungi, nematodes, viruses, mollicutes (mycoplasmas, spiroplasmas), protozoa, phanerogams; rickettsias, and viroids, insects and parasitic plants.

Abiotic stress means "sub-optimal growing condition", referring to environmental stress, damages by weather and other environmental factors, limited water and nutrient availability and sub-optimal disposability. Limited water availability can induce drought, heat, cold or salt stress.

[0005.1.1.1] Plants infectious diseases, in other words biotic stress, are responsible for significant crop losses worldwide, resulting from both infection of growing plants and destruction of harvested crops. Crop losses and crop yield losses of major crops such as rice, maize (corn) and wheat caused by these stresses represent a significant economic and political factor and contribute to food shortages worldwide.

[0006.1.1.1] Agricultural biotechnology has attempted to meet humanity's growing needs through genetic modifications of plants that could increase crop yield, for example, by conferring better tolerance to biotic and abiotic stress or by increasing biomass.

The plants' natural defense mechanisms against pathogens are frequently insufficient. At the moment many genetical and biotechnological approaches are known in order to obtain plants growing under conditions of biotic stress.

[0007.1.1.1] The introduction of foreign genes from plants, animals or microbial sources can increase the defenses. Examples are the protection against feeding damage by insects by expressing *Bacillus thuringiensis* endotoxins (Vaeck et al. (1987) Nature 328:33-37) or the protection against fungal infection by expressing a bean chitinase (Broglie et al. (1991) Science 254:1194-1197).

[0008.1.1.1] Most pathogens are host-specific to a particular plant species, genus or family. For instance, blackspot of rose will not attack marigolds or lettuce. Therefore, most of the approaches described only offer resistance to a single pathogen or a narrow spectrum of pathogens.

Plant-pathogen interactions are sometimes controlled by specific interactions between avirulence genes of pathogens and gene-for-gene disease resistance (R) genes of plants. Many authors have reported the enhancement of disease resistance by transgenic approaches, as increasing the expression of the disease resistance (R) genes of plants. The characteristic defence reaction of R-gene mediated resistance is the hypersensitive response (HR), a strong resistance reaction comprising a programmed cell death of the attacked plant cell. Small antimicrobial peptides play an important role as part of the natural defense systems of plants against infectious microorganisms. Antimicrobial peptides are usually small, cationic, and amphipathic and have open-chain forms. Various types of antimicrobial peptides have been identified in plants, including thionins, maize zeamatin, coffee circulin, and wheat puroindoline and plant defensins (Kawata et. al, JARQ 37 (2), 71 – 76 (2003)).

[0009.1.1.1] Only a few approaches exist which impart a resistance to a broader spectrum of pathogens to plants. Systemic acquired resistance (SAR)--a defense mechanism in a variety of plant/pathogen interactions --can be conferred by the application of endogenous messenger substances such as jasmonic acid (JA) or salicylic acid (SA) (Ward, et al. (1991) Plant Cell 3:1085-1694; Uknes, et al. (1992) Plant Cell 4(6):645-656). Similar effects can also be achieved by synthetic compounds such as 2,6-dichloroisonicotinic acid (INA) or S-methyl benzo(1,2,3)thiadiazole-7-thiocarboxylate (BTH; Bion200) (Friedrich et al. (1996) Plant J 10(1) :61-70; Lawton et al. (1996) Plant J. 10:71-82). The expression of pathogenesis-related (PR) proteins, which are upregulated in the case of SAR, may also cause pathogen resistance in some cases.

[0010.1.1.1] In barley, the loss of the Mlo gene causes an increased and, above all, race-unspecific resistance against a large number of mildew species (Buschges R et al. (1997) Cell 88:695-705; Jorgensen J H (1977) Euphytica 26:55-62; Lyngkjaer M F et al. (1995) Plant Pathol 44:786-790). *mlo*-like resistances in other plants, in particular

in cereal species, are not described. Various methods using the Mlo gene and homologs from other cereal species for obtaining a pathogen resistance are described (WO 98/04586; WO 00/01722; WO 99/47552). The disadvantage is that the *mlo*-mediated defense mechanism comprises a spontaneous die-off of leaf cells (Wolter M et al. (1993) Mol Gen Genet 239: 122-128). Another disadvantage is that the *mlo*-deficient genotypes show hypersensitivity to hemibiotrophic pathogens such as *Magnaporthe grisea* (*M. grisea*) and *Cochliobolus sativus* (*Bipolaris sorokiniana*) (Jarosch B et al. (1999) Mol Plant Microbe Interact 12:508-514; Kumar J et al. (2001) Phytopathology 91:127-133).

10 [0011.1.1.1] The liberation of reactive oxygen species (ROS) is ascribed an important protection function in the reaction on plant pathogens (Wojtaszek P (1997). It has been shown that mutations in the catalytic subunit of NADPH oxidase in *Arabidopsis thaliana* show a reduced accumulation of reactive oxygen intermediates (ROI). With regard to the hypersensitive reaction (HR), the results were heterogeneous: while infection with the avirulent and bacterium *Pseudomonas syringae* showed a reduced HR in a double mutant, the virulent oomycete *Peronospora parasitica* showed an increased HR. (Torres M A et al. (2002) Proc Natl Acad Sci USA 99:517-522). In US 20080047033 methods for generating or increasing a pathogen resistance in plants by reducing the expression, activity or function of an NADPH oxidase is disclosed.

20 [0012.1.1.1] An other approach is directed to *pathogenesis-related proteins* (*PRPs*), in particular to the constitutive expression in transgenic plants of DNA sequences which encode the *PRPs*. The pathogenesis-related proteins are plant proteins induced following infection by a pathogen. Such *PRPs* are disclosed in US 20080120747 or US 20080090294, whereby the species of origin of these nematocidal and antifungal polypeptides are plant species. Further plant genes involved in plant resistance are known from US 7,320,892; US 20080184397 or US 20070226839 for example.

25 [0013.1.1.1] With view to the fact that plant disease constitutes a major and ongoing threat to human food stocks and animal feed, a need exists to identify methods conferring a resistance and/or tolerance to a broad spectrum of plant diseases. Most ideal such a resistance and/or tolerance should be conferred by a protein, which can be formed by plant cells directly by translation of at least one gene, two or more or combination of genes.

30 [0014.1.1.1] Accordingly, in a first embodiment, the present invention provides a method for producing a transgenic plant cell with these traits by placing the "biotic stress related protein" BSRP at disposal.

35 [0015.1.1.1] Some approaches which impart a resistance to a broader spectrum of pathogens to plants are based on enhanced tolerance to abiotic environmental factors that cause a plant to be stressed. This stress may result in the plant's gradual decline. Decline results in the plant being more susceptible to infectious diseases. So the real

40

cause of a problem is the abiotic stress factors, with the infectious disease simply being a secondary factor.

[0016.1.1.1] There is still a need to identify genes coding for polypeptides with a activity, which, when generated or increased, confers increased biotic stress resistance in plants or plant cells as compared to a corresponding non-transformed wild type control not only under conditions of abiotic stress. The increased biotic stress resistance should be directed primarily and straight to the biotic stress factor.

[0017.1.1.1] Accordingly, in one embodiment, the present invention provides the “disease resistance conferring protein” BSRP which confers increased biotic stress resistance primarily and straight to the biotic stress factor.

[0018.1.1.1] Biotic stress is caused and induced by pathogens.

[0019.1.1.1] A large group of plant pathogens of agro-economical importance are fungi. Fungi are responsible for devastating epidemics which can result in substantial losses of crops of various plant species. Fungal infection of crop plants has repeatedly resulted in catastrophic harvest failures that have caused major economic and social problems in the affected countries. Among the causal agents of infectious crop plant diseases, phytopathogenic fungi play a dominant role. The potential for serious crop epidemics still persists today. In addition to causing food shortages, fungal infection of plants can directly affect the health of humans and livestock through poisoning by toxins.

The classes with plant disease-causing fungi are Plasmodiophoromycetes, Chytridiomycetes, Zygomycetes, Oomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes.

Additionally, many fungal species may be significant as potential contaminants of food.

From the genus of ascomycete fungi *Alternaria* species are known as major plant pathogens. At least 20% of agricultural spoilage is caused by *Alternaria* species. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma.

Species in the genus *Alternaria* (Dematiaceae family, Hyphomycetes order, Fungi Imperfecti;

Kingdom: Fungi; Phylum: Ascomycota; Class: Euascomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Alternaria*) are very common and abundant. *Alternaria* infects the plant in the field and may contaminate wheat, sorghum, and barley. *Alternaria* species also infect various fruits and vegetables and can cause spoilage of these foods in refrigerated storage. *Alternaria* in general, have a world-wide geographic distribution.

A. brassicicola causes black spot disease (also called dark leaf spot) on virtually every important cultivated Brassica species including broccoli, cabbage, canola, and mustard. It is of worldwide economic importance resulting occasionally in 20-50% yield re-

ductions in crops such as canola, mustard or rape.

A. alternata causes foliage and pod blight of pea, leaf spot on *Calathea* spp. as well as late blight on tomato and potato.

Dark symptoms are signs of the pathogen, *Alternaria* spp., that have dark-colored mycelium and erect conidiophores that bear single or branched chains of conidia. Seed, seedlings, leaves and pods can be damaged. Infection can occur when seeds are sown in infected soils, but can also occur when the seeds themselves are infected.

Therefore, there is a need to identify safe and effective compositions and methods for controlling plant pathogens, in particular pathogenic fungi, specially from the genus *Alternaria* and for the production of plants, in particular seeds, having increased resistance to plant pathogens, and ultimately for the increased yield.

[0020.1.1.1] The present invention fulfills the need for plants that are resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control, and concomitantly, demonstrate increased yield. The transgenic plants of the present invention comprise microbial genes and their homologs that confer the phenotype of increased pathogen resistance when expressed in the plant.

[0021.1.1.1] Accordingly, in a first embodiment, the present invention provides a method for producing a transgenic plant cell, a plant or a part thereof with increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control, wherein the method comprises the steps of:

a) introducing into a plant cell a polynucleotide encoding a "disease resistance conferring protein" BSRP from yeast and/or *Escherichia*

b) generating from the plant cell the transgenic plant expressing the polynucleotide.

In one embodiment, the present invention provides a method for producing a transgenic plant cell, a plant or a part thereof with increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control, wherein the method comprises the overexpression of microbial genes coding for BSRP.

In one embodiment, the present invention provides a method for producing a transgenic plant cell, a plant or a part thereof with increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control, wherein the method comprises the overexpression homologs of the of microbial genes coding for BSRP.

The term "increased expression" or "overexpression" as used herein means any form of expression that is additional to the original wild-type expression level.

[0022.1.1.1] In one embodiment of the invention said BSRP has an activity selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma

subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), re-combinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reduc-
 5 tase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- re-
 sponsive, transmembrane pore-generating protein mglC, YCR087W-protein,
 YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and
 YOR299W-protein.

[0023.1.1.1] In one embodiment the present invention provides a method for produc-
 10 ing a transgenic plant cell, a plant or a part thereof with increased resistance to plant
 disease and ultimately increased yield; as compared to a corresponding non-
 transformed wild type control.

In one embodiment the present invention provides a method for producing a transgenic
 plant cell, a plant or a part thereof with increased resistance and/or tolerance to stress
 15 as compared to a corresponding non-transformed wild type control.

In one embodiment the present invention provides a method for producing a transgenic
 plant cell, a plant or a part thereof with increased yield as compared to a corresponding
 non-transformed wild type control, preferably under stress conditions.

[0024.1.1.1] In one embodiment the term "increased yield" refers to any biomass
 20 increase.

In one embodiment increased yield and/or increased biomass production includes
 higher seed yield, higher photosynthesis and/or higher dry matter production.

In one embodiment of the invention the terms "increased yield", "increased biomass" or
 "increased biomass production" means that the plants exhibit an increased growth rate
 25 from the starting of first stress condition as compared to a corresponding non-
 transformed wild type plant. An increased growth rate comprises an increased in bio-
 mass production of the whole plant, an increase in biomass of the visible part of the
 plant, e.g. of stem and leaves and florescence, visible higher and larger stem.

In one embodiment increased yield and/or increased biomass production includes
 30 higher seed yield, higher photosynthesis and/or higher dry matter production.

[0024.2.1.1] Said increased yield in accordance with the present invention can
 typically be achieved by enhancing or improving, in comparison to a non-transformed
 starting or wild-type plant, one or more yield-related traits of a plant. Such yield-related
 traits of a plant the improvement of which results in increased yield comprise, without
 35 limitation, the increase of the intrinsic yield capacity of a plant, improved nutrient use
 efficiency, and/or increased stress tolerance.

According to the present invention, yield-related traits concerning an increase of the
 intrinsic yield capacity of a plant may be manifested by improving the specific (intrinsic)
 seed yield (e.g. in terms of increased seed/ grain size, increased ear number, in-

creased seed number per ear, improvement of seed filling, improvement of seed composition, embryo and/or endosperm improvements, or the like); modification and improvement of inherent growth and development mechanisms of a plant (such as plant height, plant growth rate, pod number, pod position on the plant, number of internodes, incidence of pod shatter, efficiency of nodulation and nitrogen fixation, efficiency of carbon assimilation, improvement of seedling vigour/early vigour, enhanced efficiency of germination (under stressed or non-stressed conditions), improvement in plant architecture, cell cycle modifications, photosynthesis modifications, various signalling pathway modifications, modification of transcriptional regulation, modification of translational regulation, modification of enzyme activities, and the like); and/or the like.

According to the present invention, yield-related traits concerning an improvement or increase in nutrient use efficiency of a plant may be manifested by improving a plant's general efficiency of nutrient assimilation (e.g. in terms of improvement of general nutrient uptake and/or transport, improving a plant's general transport mechanisms, assimilation pathway improvements, and the like), and/or by improving specific nutrient use efficiency of nutrients including, but not limited to, phosphorus, potassium, and nitrogen.

According to the present invention, yield-related traits concerning an improvement or increase of stress tolerance of a plant may be manifested by improving or increasing a plant's tolerance against biotic and/ or abiotic stress. In the present application, biotic stress refers generally to plant pathogens and plant pests comprising, but not limited to, fungal diseases (including oomycete diseases), viral diseases, bacterial diseases, insect infestation, nematode infestation, and the like. In the present application, abiotic stress refers generally to abiotic environmental conditions a plant is typically confronted with, including conditions which are typically referred to as "abiotic stress" conditions including, but not limited to, drought (tolerance to drought may be achieved as a result of improved water use efficiency), heat, low temperatures and cold conditions (such as freezing and chilling conditions), salinity, osmotic stress, shade, high plant density, mechanical stress, oxidative stress, and the like.

[0024.3.1.1] The term "yield" as used herein generally refers to a measurable produce from a plant, particularly a crop.

Yield and yield increase (in comparison to a non-transformed starting or wild-type plant) can be measured in a number of ways, and it is understood that a skilled person will be able to apply the correct meaning in view of the particular embodiments, the particular crop concerned and the specific purpose or application concerned.

In the preferred embodiments of the present invention described herein, an increase in yield refers to increased biomass yield, increased seed yield, and/or increased yield regarding one or more specific content(s) of a whole plant or parts thereof or plant seed(s).

In preferred embodiments, "yield" refers to biomass yield comprising dry weight biomass yield and/or freshweight biomass yield, each with regard to the aerial and/or underground parts of a plant, depending on the specific circumstances (test conditions, specific crop of interest, application of interest, and the like). In each case, biomass
5 yield may be calculated as freshweight, dry weight or a moisture adjusted basis, and on the other hand on a per plant basis or in relation to a specific area (e.g. biomass yield per acre/ square meter/ or the like).

In other preferred embodiments, "yield" refers to seed yield which can be measured by one or more of the following parameters: number of seed or number of filled seed (per
10 plant or per area (acre/ square meter/ or the like)); seed filling rate (ratio between number of filled seeds and total number of seeds); number of flowers per plant; seed biomass or total seed weight (per plant or per area (acre/square meter/ or the like); thousand kernel weight (TKW; extrapolated from the number of filled seeds counted and their total weight; an increase in TKW may be caused by an increased seed size, an
15 increased seed weight, an increased embryo size, and/or an increased endosperm); or other parameters allowing to measure seed yield. Seed yield may be determined on a dry weight or on a fresh weight basis, or typically on a moisture adjusted basis, e.g. at 15.5 percent moisture.

In further preferred embodiments, yield refers to the specific content and/or composition of a harvestable product, including, without limitation, an enhanced and/or improved sugar content or sugar composition, an enhanced or improved starch content and/or starch composition, an enhanced and/or improved oil content and/or oil composition (such as enhanced seed oil content), an enhanced or improved protein content and/or protein composition (such as enhanced seed protein content), an enhanced
20 and/or improved vitamin content and/ or vitamin composition, or the like.

In further preferred embodiments, yield refers to the specific content and/or composition of a harvestable product, preferably an enhanced and/or improved content of at least one fine chemical selected from the group consisting of:

3,4-dihydroxyphenylalanine (dopa), alanine, α -linolenic acid (c18:cis[9,12,15]3), α -
30 linolenic acid, c18:3 (c9,c12,c15), α -linolenic acid, α -tocopherol, arginine, aspartic acid, beta-carotene, beta-sitosterol, campesterol, cerotic acid (c26:0), citrulline, coenzyme q10, coenzyme q9, ferulic acid, fructose, fumarate, fumaric acid, gamma-tocopherol/ beta-tocopherol/ 2,3-dimethyl-5-phytyl-quinol, gamma-tocopherol/beta-tocopherol, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose,
35 glutamate, glyceric acid, glycerol, glycine, hexadeca-dienoic acid (c16:cis [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), homoserine, isoleucine, isopentenyl pyrophosphate, lacton of trihydroxybutyric acid, leucine, lignoceric acid (c24:0), linoleic acid (c18:cis[9,12]2), linoleic acid c18:2 (c9,c12), lutein, malic acid, methylgalactopyranosid, myo-inositol, palmitic acid (c16:0), phenylalanine, proline, putrescine, raffinose, salicylic
40 acid, serine, shikimic acid, sinapic acid, stearic acid (c18:0), succinate, succinic acid, threonine, trihydroxybutanoic acid, tryptophane, tyrosine, valine and zeaxanthin,

or any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the above mentioned fine chemicals.

In one embodiment, the yield of the transgenic plants of the invention is increased by an enhanced nitrogen use efficiency (NUE). An improvement or increase in nitrogen
5 use efficiency of a plant may be manifested by improving a plant's general efficiency of nitrogen assimilation (e.g. in terms of improvement of general nitrogen uptake and/or transport, improving a plant's general transport mechanisms, assimilation pathway im-

provements, and the like), and/or by improving specific nitrogen use efficiency.
In a preferred meaning according to the present application, "yield" as described herein
10 may also refer to the harvestable yield of a plant, which largely depends on the specific plant/ crop of interest as well as its intended application (such as food production, feed production, processed food production, biofuel, biogas or alcohol production, or the like) of interest in each particular case. Thus, yield may also be calculated as harvest index (expressed as a ratio of the weight of the respective harvestable parts divided by
15 the total biomass), harvestable parts weight per area (acre, squaremeter, or the like); and the like.

Preferably, the preferred enhanced or improved yield characteristics of a plant described herein according to the present invention can be achieved in the absence or presence of stress conditions.

20 The meaning of "yield" is, thus, mainly dependent on the crop of interest and the intended application, and it is understood, that the skilled person will understand in each particular case what is meant from the circumstances of the description.

[0025.1.1.1] As used herein, the term "stress" refers to any sub-optimal growing condition and includes biotic and abiotic stress.

25 Generally, the term "increased tolerance to stress" can be defined as survival of plants, and/or higher yield production, under stress conditions as compared to a non-transformed wild type or starting plant.

The resistance and/or tolerance to abiotic stress of the transformed cells of the invention is disclosed in WO2004/092398, which is incorporated by reference.

30 **[0026.1.1.1]** In one embodiment the term "increased yield" refers to increased yield under condition of abiotic stress as compared to a corresponding non-transformed wild type control.

Increased yield is achieved due to enhanced resistance and/or tolerance to abiotic stress as compared to a corresponding non-transformed wild type control.

35 **[0027.1.1.1]** Abiotic stress preferably includes sub-optimal conditions associated with drought, cold or salinity or combinations thereof. In preferred embodiments, abiotic stress is drought and low water content. Wherein drought stress means any environmental stress which leads to a lack of water in plants or reduction of water supply to plants.

In one embodiment of the invention the term “increased resistance to abiotic stress” relates to an increased resistance to water stress, which is produced as a secondary stress by cold, and/or salt, and/or, of course, as a primary stress during drought.

[0028.1.1.1] In one embodiment increased abiotic stress resistance refers to resistance to drought.

In the present invention, enhanced tolerance to drought may, for example and preferably, be determined according to the following method:

Transformed plants are grown in pots in a growth chamber (e.g. York, Mannheim, Germany). In case the plants are *Arabidopsis thaliana* soil is prepared as 4:1 (v/v) mixture of soil and quartz sand. Standard growth conditions are: photoperiod of 16 h light and 8 h dark, 20 °C, 60% relative humidity, and a photon flux density of 150 µE. To induce germination, sown seeds are kept at 4 °C, in the dark, for 3 days. Plants are watered daily until they were approximately 3 weeks old at which time drought was imposed by withholding water. Parallely, the relative humidity was reduced in 10% increments every second day to 20%. After approximately 12 days of withholding water, most plants show visual symptoms of injury, such as wilting and leaf browning, whereas tolerant plants can be identified as being visually turgid and healthy green in color. Plants are scored for symptoms of drought injury in comparison to neighbouring plants for 3 days in succession.

Three successive experiments can be conducted. In the first experiment, 10 independent T2 lines are sown for each gene being tested. The percentage of plants not showing visual symptoms of injury is determined. In the second experiment, the lines that are scored as tolerant in the first experiment are put through a confirmation screen according to the same experimental procedures. In this experiment, plants of each tolerant line are grown and treated as before. In the third experiment, at least 7 replicates of the most tolerant line are grown and treated as before. The average and maximum number of days of drought survival after wild-type control have visually died are determined. Additionally measurements of chlorophyll fluorescence are made in stressed and non-stressed plants using a Mini-PAM (Heinz Walz GmbH, Effeltrich, Germany).

[0029.1.1.1] In one embodiment the term “increased yield” refers to increased yield under condition of biotic stress, e.g. increased resistance and/or tolerance to plant disease, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control.

Increased yield is achieved due to enhanced resistance and/or tolerance to biotic stress as compared to a corresponding non-transformed wild type control.

[0029.2.1.1] In one embodiment the term “increased yield” refers to increased yield under condition of biotic and abiotic stress.

In further preferred embodiments, increased yield refers under stress conditions to enhanced biomass and/or the specific content and/or composition of a harvestable prod-

uct, preferably an enhanced and/or improved content of at least one fine chemical selected from the group consisting of:

- 3,4-dihydroxyphenylalanine (dopa), alanine, α -linolenic acid (c18:cis[9,12,15]3), α -linolenic acid, c18:3 (c9,c12,c15), α -linolenic acid, α -tocopherol, arginine, aspartic acid, beta-carotene, beta-sitosterol, campesterol, cerotic acid (c26:0), citrulline, coenzyme q10, coenzyme q9, ferulic acid, fructose, fumarate, fumaric acid, gamma-tocopherol/ beta-tocopherol/ 2,3-dimethyl-5-phytyl-quinol, gamma-tocopherol/beta-tocopherol, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose, glutamate, glyceric acid, glycerol, glycine, hexadeca-dienoic acid (c16:cis [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), homoserine, isoleucine, isopentenyl pyrophosphate, lacton of trihydroxybutyric acid, leucine, lignoceric acid (c24:0), linoleic acid (c18:cis[9,12]2), linoleic acid c18:2 (c9,c12), lutein, malic acid, methylgalactopyranosid, myo-inositol, palmitic acid (c16:0), phenylalanine, proline, putrescine, raffinose, salicylic acid, serine, shikimic acid, sinapic acid, stearic acid (c18:0), succinate, succinic acid, threonine, trihydroxybutanoic acid, tryptophane, tyrosine, valine and zeaxanthin, or any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the above mentioned fine chemicals.

[0030.1.1.1] Biotic stress preferably includes sub-optimal conditions associated with plant disease.

- Resistance to biotic stress refers to resistance of a plant to plant disease as compared to a corresponding non-transformed wild type control. Biotic stress and concomitantly the plant disease are caused by pathogens, that include insects, fungi, bacteria, viruses, nematodes, viroids, mycoplasmas, etc, preferably pathogenic fungi and/or nematodes.

- "Plant pathogen" refers to any agent that causes a disease state in a plant, including viruses, fungi, bacteria, nematodes, and other microorganisms.

- Resistance to biotic stress or "pathogen resistance" denotes the reduction or weakening of disease symptoms of a plant following infection by a pathogen. The symptoms can be manifold, but preferably comprise those which directly or indirectly have an adverse effect on the quality of the plant, the quantity of the yield, the suitability for use as feedstuff or foodstuff, or else which make sowing, planting, harvesting or processing of the crop difficult. Pathogen caused disease symptoms include size and/or frequency of necrotic or chlorotic lesions on plant tissue, brown lesions, often with light tan to whitish centers, with dark purplish to black outer margin, eventually with chlorotic zone surrounding the lesion; size and/or frequency of cankers, black spot disease (also called dark leaf spot), foliage, blight etc.

[0031.1.1.1] In one embodiment increased biotic stress resistance refers to resistance to plant disease, preferably pathogenic fungi.

- In the present invention, enhanced tolerance to pathogenic fungi may, for example and preferably, be determined according to the following method:

Transformed plants are grown in pots in a growth chamber (e.g. York, Mannheim, Germany). In case the plants are *Arabidopsis thaliana* nutrient rich soil (GS90, Tantau, Wansdorf, Germany) is used. Pots are filled with soil and placed into trays. Water is added to the trays to let the soil take up appropriate amount of water for the sowing procedure. The seeds for transgenic *Arabidopsis thaliana* C24 plants are sown in pots (10 cm diameter). Then the filled tray is covered with a transparent lid without holes and transferred into the shelf system of the growth chamber. Lid and pots are sprayed daily. Stratification is established for a period of 4 days in the dark at 4°C for 8 h, in the light (220 $\mu\text{mol}/\text{m}^2\text{s}$) at 20°C for 16 h, relative humidity with 68%. After 4 days the parameters are changed to 12 h light and 12 h darkness and covers are removed. In case the transgenic plants contain the bar marker gene, BASTA selection is done at day 5, 7, 10 and 12 after sowing by spraying pots with plantlets from the top. Therefore, a 0.02% (v/v) solution of BASTA concentrate (183 g/l glufosinate-ammonium) in tap water is sprayed. The location of the trays inside the chambers is changed on working days after sowing. Watering is done as necessary after covers is removed from the trays. Plants are piqued 14 days after sowing in another pot with GS90, 5 plants together in 1 pot and 3 pots per line. 4 lines and 1 wild type (in the middle) can be placed together in 1 tray. The tray is covered with the lid and sprayed for 3 days. Positive (sensitive = pad3-1-wt-F1-(1)) and negative (resistant = Col-0-Lehle-F1-(1)) controls can be in an extra tray. 28 days after sowing phenotypic information is valued in case of plants differ from the wild type control. 31 days after sowing tap water is added in the tray in a height of 1cm. A day later plants are sprayed 3 times with the fungal spore suspensions preferably *Alternaria brassicicola* or *Alternaria alternata* ($1,5 \times 10^6$ spores / ml) and covered again. Lid are sprayed 2 times a day with water for 2 days. 3 and 4 days after inoculation the evaluation were done.

The disease symptoms can be scored in comparison to untransformed wild type control plants. The range of scoring can be between 1 and 5. Leafs with symptoms of chlorotic areas and mazaration of the tissue are counted of all three pots. The phenotype (bigness and habitus) of the plants have an influence to the final scoring.

[0032.1.1.1] In one embodiment the invention provides plants with broad spectrum stress resistance as compared to a corresponding non-transformed wild type control.

In one embodiment "broad spectrum" resistance refers to enhanced resistance against at least two, three, four, or more stress conditions. Stress conditions can be abiotic and/or biotic stress. Preferably the plants according to the invention have a increased resistance to pathogenic fungi and preferably a further increased yield related trait.

In one embodiment "broad spectrum" resistance refers to enhanced resistance against at least two, three, four, or more pathogens of different pathogen species. Preferably the pathogens are selected from the group consisting of pathogenic fungi.

[0032.2.1.1] In one embodiment of the invention pathogenic fungi and the disease with which they are associated are selected from the group consisting of:

Plasmodiophoromycota such as *Plasmodiophora brassicae* (clubroot of crucifers), *Spongospora subterranea* (powdery scab of potato tubers), *Polymyxa graminis* (root disease of cereals and grasses),

Oomycota such as *Bremia lactucae* (downy mildew of lettuce), *Peronospora* (downy mildew) in snapdragon (*P. antirrhini*), onion (*P. destructor*), spinach (*P. effusa*), soybean (*P. manchurica*), tobacco ("blue mold"; *P. tabacina*), alfalfa and clover (*P. trifolium*), *Pseudoperonospora humuli* (downy mildew of hops), *Plasmopara* (downy mildew in grapevines) (*P. viticola*) and sunflower (*P. halstedii*), *Sclerophthora macrospora* (downy mildew in cereals and grasses), *Pythium* (seed rot, seedling damping-off, and root rot of all types of plants, for example damping-off of Beta beet caused by *P. debaryanum*), *Phytophthora infestans* (blight in potato, brown rot in tomato and the like), *Albugo spec.* (white rust on cruciferous plants) or *Phytophthora sojae*.

Ascomycota such as *Microdochium nivale* (snow mold of rye and wheat), *Fusarium graminearum*, *Fusarium culmorum* (partial ear sterility mainly in wheat), *Fusarium oxysporum* (*Fusarium* wilt of tomato), *Blumeria graminis* (powdery mildew of barley (f.sp. *hordei*) and wheat (f.sp. *tritici*)), *Erysiphe pisi* (powdery mildew of pea), *Nectria galligena* (*Nectria* canker of fruit trees), *Uncinula necator* (powdery mildew of grapevine), *Pseudopeziza tracheiphila* (red fire disease of grapevine), *Claviceps purpurea* (ergot on, for example, rye and grasses), *Gaeumannomyces graminis* (take-all on wheat, rye and other grasses), *Magnaporthe grisea* (rice blast disease), *Pyrenophora graminea* (leaf stripe of barley), *Pyrenophora teres* (net blotch of barley), *Pyrenophora tritici-repentis* (leaf blight of wheat), *Venturia inaequalis* (apple scab), *Sclerotinia sclerotium* (stalk break, stem rot), *Pseudopeziza medicaginis* (leaf spot of alfalfa, white and red clover) or *Mycoshaerella fijiensis* (Black Sigatoka disease), *Fusarium* rots (*Fusarium moniliforme* and *F. graminearum*) and Anthracnose stalk rot (*Colletotrichum graminicola*), *Diplodia* stalk rot (*Diplodia maydis*), charcoal stalk rot (*Macrophomina phaseolina*).

Basidiomycetes such as *Typhula incarnata* (typhula blight on barley, rye, wheat), *Ustilago maydis* (blister smut on maize), *Ustilago nuda* (loose smut on barley), *Ustilago tritici* (loose smut on wheat, spelt), *Ustilago avenae* (loose smut on oats), *Rhizoctonia solani* (*rhizoctonia* root rot of potato), *Sphacelotheca* spp. (head smut of sorghum), *Melampsora lini* (rust of flax), *Puccinia graminis* (stem rust of wheat, barley, rye, oats), *Puccinia recondita* (leaf rust on wheat), *Puccinia dispersa* (brown rust on rye), *Puccinia hordei* (leaf rust of barley), *Puccinia coronata* (crown rust of oats), *Puccinia striiformis* (yellow rust of wheat, barley, rye and a large number of grasses), *Uromyces appendiculatus* (brown rust of bean), *Sclerotium rolfsii* (root and stem rots of many plants) or *Phakopsora pachyrhizi* (soybean rust).

Deuteromycetes (Fungi imperfecti) such as *Septoria nodorum* (glume blotch) of wheat (*Septoria tritici*), *Pseudocercospora herpotrichoides* (eyespot of wheat, barley, rye), *Rynchosporium secalis* (leaf spot on rye and barley), *Alternaria solani* (early blight of potato, tomato), *Phoma betae* (blackleg on Beta beet), *Cercospora beticola* (leaf spot

on Beta beet), *Alternaria brassicae* (black spot on oilseed rape, cabbage and other crucifers), *Alternaria alternata* (*Alternaria* leaf spot; Tobacco brown spot; Apple core rot; Tomato fruit rot; Bean leaf blight; Apple storage rot; Pear storage rot), *Verticillium dahliae* (*verticillium* wilt), *Colletotrichum lindemuthianum* (bean anthracnose), *Phoma lingam* (blackleg of cabbage and oilseed rape), *Botrytis cinerea* (gray mold of grape-vine, strawberry, tomato, hops and the like).

More preferred are *Phytophthora infestans* (potato blight, brown rot in tomato and the like), *Microdochium nivale* (previously *Fusarium nivale*; snow mold of rye and wheat), *Fusarium graminearum*, *Fusarium culmorum* (partial ear sterility of wheat), *Fusarium oxysporum* (*Fusarium* wilt of tomato), *Blumeria graminis* (powdery mildew of barley (f. sp. *hordei*) and wheat (f. sp. *tritici*)), *Magnaporthe grisea* (rice blast disease), *Sclerotinia sclerotium* (stalk break, stem rot), *Septoria nodorum* and *Septoria tritici* (glume blotch of wheat), *Alternaria brassicae* (black spot of oilseed rape, cabbage and other crucifers), *Phoma lingam* (blackleg of cabbage and oilseed rape) and *Alternaria alternata* (*Alternaria* leaf spot; Tobacco brown spot; Apple core rot; Tomato fruit rot; Bean leaf blight; Apple storage rot; Pear storage rot),.

Most preferred are *Alternaria brassicae* and *Alternaria alternata*.

[0033.1.1.1] In one embodiment this invention fulfills in part the need to identify new, unique genes capable of conferring increased resistance to abiotic stress, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control, upon expression or over-expression of endogenous and/or exogenous genes.

[0034.1.1.1] Accordingly, the present invention relates to a method for producing a transgenic plant cell, a plant or a part thereof with increased resistance to biotic abiotic stress, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control, which comprises

(a) increasing or generating one or more activities selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mgIC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein. in plant cell, a plant or a part thereof, and

(b) growing the plant cell, a plant or a part thereof under conditions which permit the development of a plant with increased resistance to abiotic stress, preferably to

pathogenic fungi abiotic stress, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type plant.

[0035.1.1.1] In a preferred embodiment the present invention relates to a method for producing a transgenic plant cell, a plant or a part thereof with increased resistance to abiotic stress, preferably to pathogenic fungi abiotic stress, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises increasing or generating one or more activities of a polypeptide selected from the group consisting of:

- (i) a polypeptide comprising a polypeptide, a consensus sequence or at least one polypeptide motif as depicted in column 5 or 7 of Table II or of Table IV, respectively; or
- (ii) an expression product of a nucleic acid molecule comprising a polynucleotide as depicted in column 5 or 7 of Table I,
- (iii) or a functional equivalent of (i) or (ii).

[0036.1.1.1] In a preferred embodiment the present invention relates to a method

for producing a transgenic plant cell, a plant or a part thereof with increased resistance to abiotic stress, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises increasing or generating the expression of at least one nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of:

- (a) a nucleic acid molecule encoding the polypeptide shown in column 5 or 7 of Table II;
- (b) a nucleic acid molecule shown in column 5 or 7 of Table I;
- (c) a nucleic acid molecule, which, as a result of the degeneracy of the genetic code, can be derived from a polypeptide sequence depicted in column 5 or 7 of Table II and confers an increased resistance to biotic stress, preferably pathogenic as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
- (d) a nucleic acid molecule having at least 30 % identity with the nucleic acid molecule sequence of a polynucleotide comprising the nucleic acid molecule shown in column 5 or 7 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- (e) a nucleic acid molecule encoding a polypeptide having at least 30 % identity with the amino acid sequence of the polypeptide encoded by the nucleic acid molecule of (a) to (c) and having the activity of a polypeptide encoded by a nucleic acid molecule comprising a polynucleotide as depicted in column 5 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;

- (f) a nucleic acid molecule which hybridizes with a nucleic acid molecule of (a) to (c) under stringent hybridization conditions and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 5 (g) a nucleic acid molecule encoding a polypeptide which can be isolated with the aid of monoclonal or polyclonal antibodies made against a polypeptide encoded by one of the nucleic acid molecules of (a) to (e) and having the activity represented by the nucleic acid molecule comprising a polynucleotide as depicted in column 5 of Table I;
- 10 (h) a nucleic acid molecule encoding a polypeptide comprising the consensus sequence or one or more polypeptide motifs as shown in column 7 of Table IV and preferably having the activity represented by a polypeptide comprising a polypeptide as depicted in column 5 of Table II or IV;
- (i) a nucleic acid molecule encoding a polypeptide having the activity represented by a protein as depicted in column 5 of Table II and confers an increased resistance to
- 15 biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- (j) a nucleic acid molecule which comprises a polynucleotide, which is obtained by amplifying a cDNA library or a genomic library using the primers in column 7 of Table III and preferably having the activity represented by a polypeptide comprising a polypep-
- 20 tide as depicted in column 5 of Table II or IV;
- and
- (k) a nucleic acid molecule which is obtainable by screening a suitable nucleic acid library under stringent hybridization conditions with a probe comprising a complementary sequence of a nucleic acid molecule of (a) or (b) or with a fragment thereof, having
- 25 at least 15 nt, preferably 20 nt, 30 nt, 50 nt, 100 nt, 200 nt or 500 nt of a nucleic acid molecule complementary to a nucleic acid molecule sequence characterized in (a) to (e) and encoding a polypeptide having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II.

[0037.1.1.1] Unless otherwise noted, the terms used herein are to be understood

30 according to conventional usage by those of ordinary skill in molecular biology. In addition to the definitions of terms provided below, definitions of common terms in molecular biology may also be found in Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement).

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Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. A number of standard

molecular biology techniques are described in Sambrook and Russell, 2001 Molecular Cloning, Third Edition, Cold Spring Harbor, Plainview, New York; Sambrook et al., 1989 Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al., 1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (Ed.) 1993 Meth. Enzymol. 218, Part I; Wu (Ed.) 1979 Meth Enzymol. 68; Wu et al., (Eds.) 1983 Meth. Enzymol. 100 and 101; Grossman and Mol-
 5 dave (Eds.) 1980 Meth. Enzymol. 65; Miller (Ed.) 1972 Experiments in Molecular Ge-
 netics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Prim-
 rose, 1981 Principles of Gene Manipulation, University of California Press, Berkeley;
 10 Schleif and Wensink, 1982 Practical Methods in Molecular Biology; Glover (Ed.) 1985
 DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (Eds.) 1985 Nu-
 cleic Acid Hybridization, IRL Press, Oxford, UK; and Setlow and Hollaender 1979 Ge-
 netic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York.

[0037.2.1.1] As used herein and in the appended claims, the singular form “a”,
 15 “an”, or “the” includes plural reference unless the context clearly dictates otherwise. As
 used herein, the word “or” means any one member of a particular list and also includes
 any combination of members of that list.

The term “cell” or “plant cell” as used herein refers to single cell, and also includes a
 population of cells. The population may be a pure population comprising one cell type.
 20 Likewise, the population may comprise more than one cell type. A plant cell within the
 meaning of the invention may be isolated (e.g., in suspension culture) or comprised in
 a plant tissue, plant organ or plant at any developmental stage.

The term “tissue” with respect to a plant (or “plant tissue”) means arrangement of mul-
 tiple plant cells, including differentiated and undifferentiated tissues of plants. Plant
 25 tissues may constitute part of a plant organ (e.g., the epidermis of a plant leaf) but may
 also constitute tumor tissues (e.g., callus tissue) and various types of cells in culture
 (e.g., single cells, protoplasts, embryos, calli, protocorm-like bodies, etc.). Plant tissues
 may be in planta, in organ culture, tissue culture, or cell culture.

The term “organ” with respect to a plant (or “plant organ”) means parts of a plant and
 30 may include, but not limited to, for example roots, fruits, shoots, stems, leaves, hypo-
 cotyls, cotyledons, anthers, sepals, petals, pollen, seeds, etc.

The term “plant” as used herein can, depending on context, be understood to refer to
 whole plants, plant cells, plant organs, plant seeds, and progeny of same. The word
 “plant” also refers to any plant, particularly, to seed plant, and may include, but not lim-
 35 ited to, crop plants. Plant parts include, but are not limited to, stems, roots, shoots,
 fruits, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, gameto-
 phytes, sporophytes, pollen, microspores, hypocotyls, cotyledons, anthers, sepals, pet-
 als, pollen, seeds and the like. The term “plant” as used herein can be monocotyle-
 donous crop plants, such as, for example, cereals including wheat, barley, sorghum,
 40 rye, triticale, maize, rice, sugarcane, and trees including apple, pear, quince, plum,

cherry, peach, nectarine, apricot, papaya, mango, poplar, pine, sequoia, cedar, and oak. The term "plant" as used herein can be dicotyledonous crop plants, such as pea, alfalfa, soybean, carrot, celery, tomato, potato, cotton, tobacco, pepper, canola, oilseed rape, beet, cabbage, cauliflower, broccoli, lettuce and *Arabidopsis thaliana*. The class of plants that can be used in the method of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, horsetails, psilophytes, bryophytes, and multicellular algae. The plant can be from a genus selected from the group consisting of *Medicago*, *Solanum*, *Brassica*, *Cucumis*, *Solanum*, *Juglans*, *Gossypium*, *Malus*, *Vitis*, *Antirrhinum*, *Populus*, *Fragaria*, *Arabidopsis*, *Picea*, *Capsicum*, *Chenopodium*, *Dendranthema*, *Pharbitis*, *Pinus*, *Pisum*, *Oryza*, *Zea*, *Triticum*, *Triticale*, *Secale*, *Lolium*, *Hordeum*, *Glycine*, *Pseudotsuga*, *Kalanchoe*, *Beta*, *Helianthus*, *Nicotiana*, *Cucurbita*, *Rosa*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Raphanus*, *Sinapis*, *Atropa*, *Datura*, *Hyoscyamus*, *Nicotiana*, *Petunia*, *Digitalis*, *Majorana*, *Cia-*
horium, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargo-*
nium, *Panieum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Browaalia*, *Phaseo-*
lus, *Avena*, and *Allium*.

[0037.3.1.1] The term "transgenic" as used herein is intended to refer to cells and/or plants which contain a transgene, or whose genome has been altered by the introduction of at least one transgene, or that have incorporated exogenous genes or polynucleotides. Transgenic cells, tissues, organs and plants may be produced by several methods including the introduction of a "transgene" comprising at least one polynucleotide (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein.

The term "wild type" as used herein refers to a plant cell, seed, plant component, plant tissue, plant organ, or whole plant that has not been genetically modified or treated in an experimental sense.

The term "control plant" as used herein refers to a plant cell, an explant, seed, plant component, plant tissue, plant organ, or whole plant used to compare against transgenic or genetically modified plant for the purpose of identifying an enhanced phenotype or a desirable trait in the transgenic or genetically modified plant. A "control plant" may in some cases be a transgenic plant line that comprises an empty vector or marker gene, but does not contain the recombinant polynucleotide of interest that is present in the transgenic or genetically modified plant being evaluated. A control plant may be a plant of the same line or variety as the transgenic or genetically modified plant being tested, or it may be another line or variety, such as a plant known to have a specific phenotype, characteristic, or known genotype. A suitable control plant would include a genetically unaltered or non-transgenic plant of the parental line used to generate a transgenic plant herein.

The term "non-transformed wild type control" according to the invention is a wild type which was genetically modified or treated by the method of the invention, so that it can be used as a control for the plant cell of the invention.

[0037.4.1.1] In accordance with the invention, the plant may be a plant selected from the group consisting of monocotyledonous plants and dicotyledonous plants. The plant can be from a genus selected from the group consisting of maize, wheat, rice, barley, oat, rye, sorghum, banana, and ryegrass. The plant can be from a genus selected from the group consisting of pea, alfalfa, soybean, carrot, celery, tomato, potato, cotton, tobacco, pepper, oilseed rape, beet, cabbage, cauliflower, broccoli, lettuce and *Arabidopsis thaliana*.

[0037.5.1.1] The present invention also provides a transgenic seed which is true breeding for a polynucleotide described above, parts from the transgenic plant described above, and progeny plants from such a plant, including hybrids and inbreds. The invention also provides a method of plant breeding, e.g., to develop or propagate a crossed transgenic plant. The method comprises crossing a transgenic plant comprising a particular expression vector of the invention with itself or with a second plant, e.g., one lacking the particular expression vector, and harvesting the resulting seed of a crossed plant whereby the harvested seed comprises the particular expression vector. The seed is then planted to obtain a crossed transgenic progeny plant. The plant may be a monocot or a dicot. The crossed transgenic progeny plant may have the particular expression vector inherited through a female parent or through a male parent. The second plant may be an inbred plant. The crossed transgenic plant may be an inbred or a hybrid. Also included within the present invention are seeds of any of these crossed transgenic plants and their progeny.

[0038.1.1.1] Accordingly, the present invention relates to a method for producing a transgenic plant cell, a plant or a part thereof with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises

(a) increasing or generating one or more activities selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl-responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein. in the plastid of a plant cell,

and

(b) growing the plant cell under conditions which permit the development of a plant with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant.

5 [0039.1.1.1] In one embodiment the present invention relates to a method for producing a transgenic plant cell, a plant or a part thereof with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises

10 (a) increasing or generating the activity of a protein as shown in table II, column 3 encoded by the nucleic acid sequences as shown in table I, column 5 or 7, in the plastid of a plant cell,

and

15 (b) growing the plant cell under conditions which permit the development of a plant with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant.

[0040.1.1.1] In another embodiment the present invention is related to a method for producing a transgenic plant cell, a plant or a part thereof with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises

20 a. increasing or generating one or more activities selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein. in an organelle of a plant cell or

25 b. increasing or generating the activity of a protein as shown in table II, column 3 encoded by the nucleic acid sequences as shown in table I, column 5 or 7, which are joined to a nucleic acid sequence encoding a transit peptide in a plant cell; or

30 c. increasing or generating the activity of a protein as shown in table II, column 3 encoded by the nucleic acid sequences as shown in table I, column 5 or 7, which are

joined to a nucleic acid sequence encoding chloroplast localization sequence, in a plant cell, and

- d. growing the plant cell under conditions which permit the development of a plant with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant.

[0041.1.1.1] In another embodiment, the present invention relates to a method for producing a transgenic plant cell, a plant or a part thereof with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant cell, a plant or a part thereof, which comprises

- a) increasing or generating the activity of a protein as shown in table II, column 3 encoded by the nucleic acid sequences as shown in table I, column 5 or 7, in an organelle of a plant through the transformation of the organelle, or
- b) increasing or generating the activity of a protein as shown in table II, column 3 encoded by the nucleic acid sequences as shown in table I, column 5 or 7 in the plastid of a plant, or in one or more parts thereof through the transformation of the plastids; and
- c) growing the plant cell under conditions which permit the development of a plant with increased tolerance and/or resistance to environmental stress and increased biomass production as compared to a corresponding non-transformed wild type plant.

[0042.1.1.1] In principle the nucleic acid sequence encoding a transit peptide can be isolated from every organism such as microorganisms such as algae or plants containing plastids preferably chloroplasts. A "transit peptide" is an amino acid sequence, whose encoding nucleic acid sequence is translated together with the corresponding structural gene. That means the transit peptide is an integral part of the translated protein and forms an amino terminal extension of the protein. Both are translated as so called "preprotein". In general the transit peptide is cleaved off from the preprotein during or just after import of the protein into the correct cell organelle such as a plastid to yield the mature protein. The transit peptide ensures correct localization of the mature protein by facilitating the transport of proteins through intracellular membranes. Preferred nucleic acid sequences encoding a transit peptide are derived from a nucleic acid sequence encoding a protein finally resided in the plastid and stemming from an organism selected from the group consisting of the genera

Acetabularia, Arabidopsis, Brassica, Capsicum, Chlamydomonas, Cucurbita, Dunaliella, Euglena, Flaveria, Glycine, Helianthus, Hordeum, Lemna, Lolium, Lycopersicon, Malus, Medicago, Mesembryanthemum, Nicotiana, Oenothera, Oryza, Petunia, Phaseolus, Physcomitrella, Pinus, Pisum, Raphanus, Silene, Sinapis, Solanum, Spinacea, Stevia, Synechococcus, Triticum and Zea.

[0043.1.1.1] Advantageously such transit peptides, which are beneficially used in the inventive process, are derived from the nucleic acid sequence encoding a protein selected from the group consisting of

ribulose biphosphate carboxylase/oxygenase, 5-enolpyruvyl-shikimate-3-phosphate
 5 synthase, acetolactate synthase, chloroplast ribosomal protein CS17, Cs protein, ferredoxin, plastocyanin, ribulose biphosphate carboxylase activase, tryptophan synthase, acyl carrier protein, plastid chaperonin-60, cytochrome c_{552} , 22-kDa heat shock protein, 33-kDa Oxygen-evolving enhancer protein 1, ATP synthase γ subunit, ATP synthase δ subunit, chlorophyll-a/b-binding proteinII-1, Oxygen-evolving enhancer protein 2, Oxygen-evolving enhancer protein 3, photosystem I: P21, photosystem I: P28, photosystem I: P30, photosystem I: P35, photosystem I: P37, glycerol-3-phosphate acyltransferases, chlorophyll a/b binding protein, CAB2 protein, hydroxymethyl-bilane synthase, pyruvate-orthophosphate dikinase, CAB3 protein, plastid ferritin, ferritin, early light-inducible protein, glutamate-1-semialdehyde aminotransferase, protochlorophyllide
 15 reductase, starch-granule-bound amylase synthase, light-harvesting chlorophyll a/b-binding protein of photosystem II, major pollen allergen Lol p 5a, plastid ClpB ATP-dependent protease, superoxide dismutase, ferredoxin NADP oxidoreductase, 28-kDa ribonucleoprotein, 31-kDa ribonucleoprotein, 33-kDa ribonucleoprotein, acetolactate synthase, ATP synthase CF_0 subunit 1, ATP synthase CF_0 subunit 2, ATP synthase
 20 CF_0 subunit 3, ATP synthase CF_0 subunit 4, cytochrome f, ADP-glucose pyrophosphorylase, glutamine synthase, glutamine synthase 2, carbonic anhydrase, GapA protein, heat-shock-protein hsp21, phosphate translocator, plastid ClpA ATP-dependent protease, plastid ribosomal protein CL24, plastid ribosomal protein CL9, plastid ribosomal protein PsCL18, plastid ribosomal protein PsCL25, DAHP synthase, starch
 25 phosphorylase, root acyl carrier protein II, betaine-aldehyde dehydrogenase, GapB protein, glutamine synthetase 2, phosphoribulokinase, nitrite reductase, ribosomal protein L12, ribosomal protein L13, ribosomal protein L21, ribosomal protein L35, ribosomal protein L40, triose phosphate-3-phosphoglycerate-phosphate translocator, ferredoxin-dependent glutamate synthase, glyceraldehyde-3-phosphate dehydrogenase,
 30 NADP-dependent malic enzyme and NADP-malate dehydrogenase.

[0043.2.1.1] More preferred the nucleic acid sequence encoding a transit peptide is derived from a nucleic acid sequence encoding a protein finally resided in the plastid and stemming from an organism selected from the group consisting of the species:

Acetabularia mediterranea, Arabidopsis thaliana, Brassica campestris, Brassica napus,
 35 Capsicum annuum, Chlamydomonas reinhardtii, Cururbita moschata, Dunaliella salina, Dunaliella tertiolecta, Euglena gracilis, Flaveria trinervia, Glycine max, Helianthus annuus, Hordeum vulgare, Lemna gibba, Lolium perenne, Lycopersion esculentum, Malus domestica, Medicago falcata, Medicago sativa, Mesembryanthemum crystallinum, Nicotiana plumbaginifolia, Nicotiana sylvestris, Nicotiana tabacum, Oenothera hookeri,
 40 Oryza sativa, Petunia hybrida, Phaseolus vulgaris, Physcomitrella patens, Pinus tunbergii, Pisum sativum, Raphanus sativus, Silene pratensis, Sinapis alba, Solanum

tuberosum, Spinacea oleracea, Stevia rebaudiana, Synechococcus, Synechocystis, Triticum aestivum and Zea mays.

[0043.3.1.1] Even more preferred nucleic acid sequences are encoding transit peptides as disclosed by von Heijne et al. [Plant Molecular Biology Reporter, Vol. 9 (2), 1991: 104 – 126], which are hereby incorporated by reference. Table V shows some examples of the transit peptide sequences disclosed by von Heijne et al. According to the disclosure of the invention especially in the examples the skilled worker is able to link other nucleic acid sequences disclosed by von Heijne et al. to the nucleic acid sequences shown in table I, columns 5 and 7. Most preferred nucleic acid sequences encoding transit peptides are derived from the genus Spinacia such as chloroplast 30S ribosomal protein PSrp-1, root acyl carrier protein II, acyl carrier protein, ATP synthase: γ subunit, ATP synthase: δ subunit, cytochrom f, ferredoxin I, ferredoxin NADP oxidoreductase (= FNR), nitrite reductase, phosphoribulokinase, plastocyanin or carbonic anhydrase. The skilled worker will recognize that various other nucleic acid sequences encoding transit peptides can easily isolated from plastid-localized proteins, which are expressed from nuclear genes as precursors and are then targeted to plastids. Such transit peptides encoding sequences can be used for the construction of other expression constructs. The transit peptides advantageously used in the inventive process and which are part of the inventive nucleic acid sequences and proteins are typically 20 to 120 amino acids, preferably 25 to 110, 30 to 100 or 35 to 90 amino acids, more preferably 40 to 85 amino acids and most preferably 45 to 80 amino acids in length and functions post-translationally to direct the protein to the plastid preferably to the chloroplast. The nucleic acid sequences encoding such transit peptides are localized upstream of nucleic acid sequence encoding the mature protein. For the correct molecular joining of the transit peptide encoding nucleic acid and the nucleic acid encoding the protein to be targeted it is sometimes necessary to introduce additional base pairs at the joining position, which forms restriction enzyme recognition sequences useful for the molecular joining of the different nucleic acid molecules. This procedure might lead to very few additional amino acids at the N-terminal of the mature imported protein, which usually and preferably do not interfere with the protein function. In any case, the additional base pairs at the joining position which forms restriction enzyme recognition sequences have to be chosen with care, in order to avoid the formation of stop codons or codons which encode amino acids with a strong influence on protein folding, like e.g. proline. It is preferred that such additional codons encode small structural flexible amino acids such as glycine or alanine.

[0044.1.1.1] As mentioned above the nucleic acid sequences coding for the proteins as shown in table II, column 3 and its homologs as disclosed in table I, columns 5 and 7 can be joined to a nucleic acid sequence encoding a transit peptide. This nucleic acid sequence encoding a transit peptide ensures transport of the protein to the plastid. The nucleic acid sequence of the gene to be expressed and the nucleic acid sequence encoding the transit peptide are operably linked. Therefore the transit peptide is fused

in frame to the nucleic acid sequence coding for proteins as shown in table II, column 3 and its homologs as disclosed in table I, columns 5 and 7.

- [0045.1.1.1]** The term “organelle” according to the invention shall mean for example “mitochondria” or preferably “plastid” (throughout the specification the “plural” shall comprise the “singular” and vice versa). The term “plastid” according to the invention are intended to include various forms of plastids including proplastids, chloroplasts, chromoplasts, gerontoplasts, leucoplasts, amyloplasts, elaioplasts and etioplasts preferably chloroplasts. They all have as a common ancestor the aforementioned proplastids.
- [0045.2.1.1]** Other transit peptides are disclosed by Schmidt et al. [J. Biol. Chem., Vol. 268, No. 36, 1993: 27447 - 27457], Della-Cioppa et al. [Plant. Physiol. 84, 1987: 965 - 968], de Castro Silva Filho et al. [Plant Mol. Biol., 30, 1996: 769 - 780], Zhao et al. [J. Biol. Chem. Vol. 270, No. 11, 1995: 6081 - 6087], Römer et al. [Biochem. Biophys. Res. Commun., Vol. 196, No. 3, 1993 : 1414 - 1421], Keegstra et al. [Annu. Rev. Plant Physiol. Plant Mol. Biol., 40, 1989: 471 - 501], Lubben et al. [Photosynthesis Res., 17, 1988: 173 - 194] and Lawrence et al. [J. Biol. Chem., Vol. 272, No. 33, 1997: 20357 - 20363]. A general review about targeting is disclosed by Kermode Allison R. in Critical Reviews in Plant Science 15 (4): 285 – 423 (1996) under the title “Mechanisms of Intracellular Protein Transport and Targeting in Plant Cells.”
- [0045.3.1.1]** Favored transit peptide sequences, which are used in the inventive process and which forms part of the inventive nucleic acid sequences are generally enriched in hydroxylated amino acid residues (serine and threonine), with these two residues generally constituting 20 – 35 % of the total. They often have an amino-terminal region empty of Gly, Pro, and charged residues. Furthermore they have a number of small hydrophobic amino acids such as valine and alanine and generally acidic amino acids are lacking. In addition they generally have a middle region rich in Ser, Thr, Lys and Arg. Overall they have very often a net positive charge.
- [0046.1.1.1]** Alternatively, nucleic acid sequences coding for the transit peptides may be chemically synthesized either in part or wholly according to structure of transit peptide sequences disclosed in the prior art. Said natural or chemically synthesized sequences can be directly linked to the sequences encoding the mature protein or via a linker nucleic acid sequence, which may be typically less than 500 base pairs, preferably less than 450, 400, 350, 300, 250 or 200 base pairs, more preferably less than 150, 100, 90, 80, 70, 60, 50, 40 or 30 base pairs and most preferably less than 25, 20, 15, 12, 9, 6 or 3 base pairs in length and are in frame to the coding sequence. Furthermore favorable nucleic acid sequences encoding transit peptides may comprise sequences derived from more than one biological and/or chemical source and may include a nucleic acid sequence derived from the amino-terminal region of the mature protein, which in its native state is linked to the transit peptide. In a preferred embodiment of the invention said amino-terminal region of the mature protein is typically less than 150 amino acids, preferably less than 140, 130, 120, 110, 100 or 90 amino acids, more

preferably less than 80, 70, 60, 50, 40, 35, 30, 25 or 20 amino acids and most preferably less than 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 amino acids in length. But even shorter or longer stretches are also possible. In addition target sequences, which facilitate the transport of proteins to other cell compartments such as the vacuole, endoplasmic reticulum, golgi complex, glyoxysomes, peroxisomes or mitochondria may be also part of the inventive nucleic acid sequence. The proteins translated from said inventive nucleic acid sequences are a kind of fusion proteins that means the nucleic acid sequences encoding the transit peptide for example the ones shown in table V, preferably the last one of the table are joint to the nucleic acid sequences shown in table I, columns 5 and 7. The person skilled in the art is able to join said sequences in a functional manner. Advantageously the transit peptide part is cleaved off from the protein part shown in table II, columns 5 and 7 during the transport preferably into the plastids. All products of the cleavage of the preferred transit peptide shown in the last line of table V have preferably the N-terminal amino acid sequences QIA CSS or QIA EFQLTT in front of the start methionine of the protein mentioned in table II, columns 5 and 7. Other short amino acid sequences of an range of 1 to 20 amino acids preferable 2 to 15 amino acids, more preferable 3 to 10 amino acids most preferably 4 to 8 amino acids are also possible in front of the start methionine of the protein mentioned in table II, columns 5 and 7. In case of the amino acid sequence QIA CSS the three amino acids in front of the start methionine are stemming from the LIC (= ligation independent cloning) cassette. Said short amino acid sequence is preferred in the case of the expression of E. coli genes. In case of the amino acid sequence QIA EFQLTT the six amino acids in front of the start methionine are stemming from the LIC cassette. Said short amino acid sequence is preferred in the case of the expression of S. cerevisiae genes. The skilled worker knows that other short sequences are also useful in the expression of the genes mentioned in table I, columns 5 and 7. Furthermore the skilled worker is aware of the fact that there is not a need for such short sequences in the expression of the genes.

Table V: Examples of transit peptides disclosed by von Heijne et al.

Trans Pep	Organism	Transit Peptide	SEQ ID NO:	Reference
1	Acetabularia mediterranea	MASIMMNKSVVLSKECAKPLATPK VTLNKRGFATTIATKNREMMVWQP FNNKMFETFSFLPP	19	Mol. Gen. Genet. 218:445-452(1989)
2	Arabidopsis thaliana	MAASLQSTATFLQSAKIATAPSRG SSHLRSTQAVGKSFGLETSSARLT CSFQSDFKDFTGKCSDAVKIAGFA	20	EMBO J. 8:3187-3194(1989)

Trans Pep	Organism	Transit Peptide	SEQ ID NO:	Reference
		LATSALVVSGASAEGAPK		)
3	Arabidopsis thaliana	MAQVSRICNGVQNPSLICNLSKSS QRKSPLSVSLKTQQHPRAYPISSS WGLKKSGMTLIGSELRLPKVMSSV STAEKASEIVLQPIREISGLIKLP	21	Mol. Gen. Genet. 210: 437 – 442 (1987)
4	Arabidopsis thaliana	MAAATTTTTTSSSISFSTKPSPSS SKSPLPISRFLPFSLNPNKSSSS SRRRGIKSSSP SS ISAVLNTTTNV TTTPSPTKPTKPETF ISRFAPDQP RKGA	22	Plant Physiol. 85:1110- 1117 (1987)
5	Arabidopsis thaliana	MITSSLTCSLQALKLSSPFAHGST PLSSLSKPNSFPNHRMPALVPV	23	J. Biol. Chem. 265:2763- 2767 (1990)
6	Arabidopsis thaliana	MASLLGTSSSAIWASPSLSSPSSK PSSSPICFRPGKLFGLSKLNAGIQI RPKKNRSRYHVSVMNVATEINSTE QVVGKFDSKKSARPVYPFAAI	24	EMBO J. 9:1337- 1346 (1990)
7	Arabidopsis thaliana	MASTALSSAIVGTSFIRRSPAPISL RSLPSANTQSLFGLKSGTARGG RVVAM	25	Plant Physiol. 93: 572- 577 (1990)
8	Arabidopsis thaliana	MAASTMALSSPAFAGKAVNLSPAA SEVLGSGRVTNRKTV	26	Nucl. Ac- ids Res. 14: 4051 – 4064 (1986)

Trans Pep	Organism	Transit Peptide	SEQ ID NO:	Reference
9	Arabidopsis thaliana	MAAITSATVTIPSFTGLKLAVSSK PKTLSTISRSSSATRAPPKLALKS SLKDFGVIAVATAASIVLAGNAMA MEVLLGSDDGSLAFVPSEFT	27	Gene 65: 59 – 69 (1988)
10	Arabidopsis thaliana	MAAAVSTVGAINRAPLSLNGSGSG AVSAPASTFLGKKVTVSRFAQSN KKSNGSFKVLAVKEDKQTDGDRWR GLAYDTSDDQIDI	28	Nucl. Ac- ids Res. 17: 2871 (1989)
11	Arabidopsis thaliana	MkSSMLSSTAWTSPAQATMVAPF TGLKSSASFPVTRKANNDITSITS NGGRVSC	29	Plant Mol. Biol. 11: 745 – 759 (1988)
12	Arabidopsis thaliana	MAASGTSATFRASVSSAPSSSSQL THLKSPFKAVKY TPLPS SRSKSSS FSVSCTIAKDPPVLMAAGSDPALW QRPDSFGRFGKFGGKYVPE	30	Proc. Natl. Acad. Sci. USA, 86: 4604 – 4608 (1989)
13	Brassica campestris	MSTTFCSSVCMQATSLAATTRISF QKPALVSTTNLSFNLRRSIPTRFS ISCAAKPETVEKVSKIVKKQLSLK DDQKVVAE	31	Nucl. Ac- ids Res. 15: 7197 (1987)
14	Brassica napus	MATTFASVSMQATSLATTTTRISF QKPVLVSNHGRTNLSFNLSRTRLI SC	32	Eur. J. Biochem. 174: 287 – 295 (1988)
15	Chlamydomo- nas reinhardtii	MQALSSRVNIAAKPQRAQRLVVRA EEVKAAPKKEVGPKRGSLVK	33	Plant Mol. Biol. 12: 463 – 474 (1989)

Trans Pep	Organism	Transit Peptide	SEQ ID NO:	Reference
16	Cucurbita mo- schata	MAELIQDKESAQSAATAAAASSGY ERRNEPAHSRKFLFVRSEEELL- SCIKK	32	FEBS Lett. 238: 424 – 430 (1988)
17	Spinacea ol- eracea	MSTINGCLTSISPSRTQLKNTSTL RPTFIANSRVNPSSSVPPSLIRNQ PVFAAPAPIITPTL	34	J. Biol. Chem. 265: 105414 – 5417 (1990)
18	Spinacea ol- eracea	MTTAVTAAVSFPSTKTTLSARCS SVISPDKISYKKVPLYYRNVSATG KMGPIRAQIASDVEAPPPAPAK- VEKMS	35	Curr. Genet. 13: 517 – 522 (1988)
19	Spinacea ol- eracea	MTTAVTAAVSFPSTKTTLSARSS SVISPDKISYKKVPLYYRNVSATG KMGPIRA	36	

[0046.2.1.1] Alternatively to the targeting of the sequences shown in table II, columns 5 and 7 preferably of sequences in general encoded in the nucleus with the aid of the targeting sequences mentioned for example in table V alone or in combination with other targeting sequences preferably into the plastids, the nucleic acids of the invention can directly be introduced into the plastidal genome. Therefore in a preferred embodiment the nucleic acid sequences shown in table I, columns 5 and 7 are directly introduced and expressed in plastids.

The term "introduced" in the context of this specification shall mean the insertion of a nucleic acid sequence into the organism by means of a "transfection", "transduction" or preferably by "transformation".

A plastid, such as a chloroplast, has been "transformed" by an exogenous (preferably foreign) nucleic acid sequence if nucleic acid sequence has been introduced into the plastid that means that this sequence has crossed the membrane or the membranes of the plastid. The foreign DNA may be integrated (covalently linked) into plastid DNA making up the genome of the plastid, or it may remain unintegrated (e.g., by including a chloroplast origin of replication). "Stably" integrated DNA sequences are those, which

are inherited through plastid replication, thereby transferring new plastids, with the features of the integrated DNA sequence to the progeny.

[0047.1.1.1] For expression a person skilled in the art is familiar with different methods to introduce the nucleic acid sequences into different organelles such as the preferred plastids. Such methods are for example disclosed by Pal Maiga (Annu. Rev. Plant Biol., 2004, 55: 289 – 313), Thomas Evans (WO 2004/040973), Kevin E. McBride et al. (US 5,455,818), Henry Daniell et al. (US 5,932,479 and US 5,693,507) and Jeffrey M. Straub et al. (US 6,781,033). A preferred method is the transformation of microspore-derived hypocotyl or cotyledonary tissue (which are green and thus contain numerous plastids) leaf tissue and afterwards the regeneration of shoots from said transformed plant material on selective medium. As methods for the transformation bombarding of the plant material or the use of independently replicating shuttle vectors are well known by the skilled worker. But also a PEG-mediated transformation of the plastids or Agrobacterium transformation with binary vectors is possible. Useful markers for the transformation of plastids are positive selection markers for example the chloramphenicol-, streptomycin-, kanamycin-, neomycin-, amikamycin-, spectinomycin-, triazine- and/or lincomycin-resistance genes. As additional markers named in the literature often as secondary markers, genes coding for the resistance against herbicides such as phosphinothricin (= glufosinate, BASTATM, LibertyTM, encoded by the bar gene), glyphosate (= N-(phosphonomethyl)glycine, Roundup ReadyTM, encoded by the 5-enolpyruvylshikimate-3-phosphate synthase gene = epsps), sulfonyleurea (= StapleTM, encoded by the acetolactate synthase gene), imidazolinone [= IMI, imazethapyr, imazamox, ClearfieldTM, encoded by the acetohydroxyacid synthase (AHAS) gene, also known as acetolactate synthase (ALS) gene] or bromoxynil (= BuctrilTM, encoded by the oxy gene) or genes coding for antibiotics such as hygromycin or G418 are useful for further selection. Such secondary markers are useful in the case when most genome copies are transformed. In addition negative selection markers such as the bacterial cytosine deaminase (encoded by the codA gene) are also useful for the transformation of plastids.

To increase the possibility of identification of transformants it is also desirable to use reporter genes other than the aforementioned resistance genes or in addition to said genes. Reporter genes are for example β -galactosidase-, β -glucuronidase- (GUS), alkaline phosphatase- and/or green-fluorescent protein-genes (GFP).

[0047.2.1.1] For the inventive process it is of great advantage that by transforming the plastids the intraspecies specific transgene flow is blocked, because a lot of species such as corn, cotton and rice have a strict maternal inheritance of plastids. By placing the genes specified in table I, columns 5 and 7 or active fragments thereof in the plastids of plants, these genes will not be present in the pollen of said plants. A further preferred embodiment of the invention relates to the use of so called "chloroplast localization sequences", in which a first RNA sequence or molecule is capable of transporting or "chaperoning" a second RNA sequence, such as a RNA sequence tran-

scribed from the sequences depicted in table I, columns 5 and 7 or a sequence encoding a protein, as depicted in table II, columns 5 and 7, from an external environment inside a cell or outside a plastid into a chloroplast. In one embodiment the chloroplast localization signal is substantially similar or complementary to a complete or intact viroid sequence. The chloroplast localization signal may be encoded by a DNA sequence, which is transcribed into the chloroplast localization RNA. The term "viroid" refers to a naturally occurring single stranded RNA molecule (Flores, C R Acad Sci III. 2001 Oct; 324(10):943-52). Viroids usually contain about 200-500 nucleotides and generally exist as circular molecules. Examples of viroids that contain chloroplast localization signals include but are not limited to ASBVd, PLMVd, CChMVd and ELVd. The viroid sequence or a functional part of it can be fused to the sequences depicted in table I, columns 5 and 7 or a sequence encoding a protein, as depicted in table II, columns 5 and 7 in such a manner that the viroid sequence transports a sequence transcribed from a sequence as depicted in table I, columns 5 and 7 or a sequence encoding a protein as depicted in table II, columns 5 and 7 into the chloroplasts. A preferred embodiment uses a modified ASBVd (Navarro et al., Virology. 2000 Mar 1;268(1):218-25).

[0048.1.1.1] In a further specific embodiment the protein to be expressed in the plastids such as the proteins depicted in table II, columns 5 and 7 are encoded by different nucleic acids. Such a method is disclosed in WO 2004/040973, which shall be incorporated by reference. WO 2004/040973 teaches a method, which relates to the translocation of an RNA corresponding to a gene or gene fragment into the chloroplast by means of a chloroplast localization sequence. The genes, which should be expressed in the plant or plants cells, are split into nucleic acid fragments, which are introduced into different compartments in the plant e.g. the nucleus, the plastids and/or mitochondria. Additionally plant cells are described in which the chloroplast contains a ribozyme fused at one end to an RNA encoding a fragment of a protein used in the inventive process such that the ribozyme can trans-splice the translocated fusion RNA to the RNA encoding the gene fragment to form and as the case may be reunite the nucleic acid fragments to an intact mRNA encoding a functional protein for example as disclosed in table II, columns 5 and 7.

[0049.1.1.1] In a preferred embodiment of the invention the nucleic acid sequences as shown in table I, columns 5 and 7 used in the inventive process are transformed into plastids, which are metabolical active. Those plastids should preferably maintain at a high copy number in the plant or plant tissue of interest, most preferably the chloroplasts found in green plant tissues, such as leaves or cotyledons or in seeds.

[0049.2.1.1] For a good expression in the plastids the nucleic acid sequences as shown in table I, columns 5 and 7 are introduced into an expression cassette using a preferably a promoter and terminator, which are active in plastids preferably a chloroplast promoter. Examples of such promoters include the psbA promoter from the gene from spinach or pea, the rbcL promoter, and the atpB promoter from corn.

[00497.3.1.1] For the purposes of the description of the present invention, the terms "cytoplasmic" and "non-targeted" are exchangeable and shall indicate, that the nucleic acid of the invention is expressed without the addition of an non-natural transit peptide encoding sequence. A non-natural transit peptide encoding sequence is a sequence
 5 which is not a natural part of a nucleic acid of the invention, e.g. of the nucleic acids depicted in table I column 5 or 7, but is rather added by molecular manipulation steps as for example described in the example under "plastid targeted expression". Therefore the terms "cytoplasmic" and "non-targeted" shall not exclude a targeted localisation to any cell compartment for the products of the inventive nucleic acid sequences by their
 10 naturally occurring sequence properties within the background of the transgenic organism. The subcellular location of the mature polypeptide derived from the enclosed sequences can be predicted by a skilled person for the organism (plant) by using software tools like TargetP (Emanuelsson et al., (2000), Predicting subcellular localization of proteins based on their N-terminal amino acid sequence., J.Mol. Biol. 300, 1005-
 15 1016.), ChloroP (Emanuelsson et al. (1999), ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites., Protein Science, 8: 978-984.) or other predictive software tools (Emanuelsson et al. (2007), Locating proteins in the cell using TargetP, SignalP, and related tools., Nature Protocols 2, 953-971).

[0049.4.1.1] Comprises/comprising and grammatical variations thereof when used in this specification are to be taken to specify the presence of stated features, integers, steps or components or groups thereof, but not to preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

In accordance with the invention, the term "plant cell" or the term "organism" as understood herein relates always to a plant cell or a organelle thereof, preferably a plastid, more preferably chloroplast.

As used herein, "plant" is meant to include not only a whole plant but also a part thereof i.e., one or more cells, and tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds.

[0050.1.1.1] Surprisingly it was found, that the transgenic expression of the *Saccharomyces cerevisiae* protein as shown in table II, column 3 and/or the transgenic expression of the *E. coli* protein as shown in table II, column 3 in a plant such as *Arabidopsis thaliana* for example, conferred to the transgenic a plant cell, a plant or a part thereof increased resistance to biotic stress, preferably pathogenic fungi as compared
 35 to a corresponding non-transformed wild type control plant cell, a plant or a part thereof

[0051.1.1.1] Accordingly, in one embodiment, in case the activity of the *Escherichia coli* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 38 or polypeptide SEQ ID NO.: 39, respectively is increased or generated, e.g. if the activi-
 40 ty of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV,

column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 38 or polypeptide SEQ ID NO.: 39, respectively is increased or generated or if the activity “glucose dehydrogenase” is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 146 or polypeptide SEQ ID NO.: 147, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 146 or polypeptide SEQ ID NO.: 147, respectively is increased or generated or if the activity “transcription regulator farR, fatty acyl- responsive” is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 165 or polypeptide SEQ ID NO.: 166, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 165 or polypeptide SEQ ID NO.: 166, respectively is increased or generated or if the activity “chloramphenicol resistance protein homolog ydeA” is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 242 or polypeptide SEQ ID NO.: 243, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 242 or polypeptide SEQ ID NO.: 243, respectively is increased or generated or if the activity “transmembrane pore-generating protein mgIC” is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 312 or polypeptide SEQ ID NO.: 313, respectively is increased or generated, e.g. if the activity of a nucleic

acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 312 or polypeptide SEQ ID NO.: 313, respectively is increased or generated or if the activity "RNA polymerase sigma-E factor (sigma-24)" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 468 or polypeptide SEQ ID NO.: 469, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 468 or polypeptide SEQ ID NO.: 469, respectively is increased or generated or if the activity "chorismate mutase-T and prephenate dehydrogenase" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 534 or polypeptide SEQ ID NO.: 535, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 534 or polypeptide SEQ ID NO.: 535, respectively is increased or generated or if the activity "recombinase A" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 970 or polypeptide SEQ ID NO.: 971, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 970 or polypeptide SEQ ID NO.: 971, respectively is increased or generated or if the activity "sulfite reductase (NADPH), flavoprotein beta subunit" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the Escherichia coli nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1143 or polypep-

5 tide SEQ ID NO.: 1144, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1143 or polypeptide SEQ ID NO.: 1144, respectively is increased or generated or if the activity "b3262-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

10 Accordingly, in one embodiment, in case the activity of the *Escherichia coli* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1174 or polypeptide SEQ ID NO.: 1175, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1174 or polypeptide SEQ ID NO.: 1175, respectively is increased or generated or if the activity "peptidyl-prolyl cis-trans isomerase A (rotamase A)" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

20 Accordingly, in one embodiment, in case the activity of the *Escherichia coli* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1521 or polypeptide SEQ ID NO.: 1522, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1521 or polypeptide SEQ ID NO.: 1522, respectively is increased or generated or if the activity "b3644-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

30 Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1660 or polypeptide SEQ ID NO.: 1661, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1660 or polypeptide SEQ ID NO.: 1661, respectively is increased or generated or if the activity "YCR087W-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

40 Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1664

or polypeptide SEQ ID NO.: 1665, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.:

5 1664 or polypeptide SEQ ID NO.: 1665, respectively is increased or generated or if the activity "YGR150c-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

10 Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1691 or polypeptide SEQ ID NO.: 1692, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II
15 or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1691 or polypeptide SEQ ID NO.: 1692, respectively is increased or generated or if the activity "YIL172C-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part
20 thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1859 or polypeptide SEQ ID NO.: 1860, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II
25 or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1859 or polypeptide SEQ ID NO.: 1860, respectively is increased or generated or if the activity "YLR168C-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part
30 thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1904 or polypeptide SEQ ID NO.: 1905, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II
35 or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1904 or polypeptide SEQ ID NO.: 1905, respectively is increased or generated or if the activity "YLR407W-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part
40 thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 1920 or polypeptide SEQ ID NO.: 1921, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1920 or polypeptide SEQ ID NO.: 1921, respectively is increased or generated or if the activity "glucose-6-phosphate 1-dehydrogenase" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 2455 or polypeptide SEQ ID NO.: 2456, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2455 or polypeptide SEQ ID NO.: 2456, respectively is increased or generated or if the activity "Gamma subunit of the translation initiation factor eIF2B" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 2477 or polypeptide SEQ ID NO.: 2478, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2477 or polypeptide SEQ ID NO.: 2478, respectively is increased or generated or if the activity "YOR299W-protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 2507 or polypeptide SEQ ID NO.: 2508, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2507 or polypeptide SEQ ID NO.: 2508, respectively is increased or generated or if the activity "ribosomal protein" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as com-

pared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred.

Accordingly, in one embodiment, in case the activity of the *Saccharomyces cerevisiae* nucleic acid molecule or a polypeptide comprising the nucleic acid SEQ ID NO.: 2683 or polypeptide SEQ ID NO.: 2684, respectively is increased or generated, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2683 or polypeptide SEQ ID NO.: 2684, respectively is increased or generated or if the activity "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" is increased or generated in an plant cell, plant or part thereof an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof is conferred..

[0052.1.1.1] For the purposes of the invention, as a rule the plural is intended to encompass the singular and vice versa.

Unless otherwise specified, the terms "polynucleotides", "nucleic acid" and "nucleic acid molecule" are interchangeably in the present context. Unless otherwise specified, the terms "peptide", "polypeptide" and "protein" are interchangeably in the present context. The term "sequence" may relate to polynucleotides, nucleic acids, nucleic acid molecules, peptides, polypeptides and proteins, depending on the context in which the term "sequence" is used. The terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid molecule(s)" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. The terms refer only to the primary structure of the molecule.

Thus, the terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid molecule(s)" as used herein include double- and single-stranded DNA and/or RNA. They also include known types of modifications, for example, methylation, "caps", substitutions of one or more of the naturally occurring nucleotides with an analog. Preferably, the DNA or RNA sequence comprises a coding sequence encoding the herein defined polypeptide.

A "coding sequence" is a nucleotide sequence, which is transcribed into an RNA, e.g. a regulatory RNA, such as a miRNA, a ta-siRNA, cosuppression molecule, an RNAi, a ribozyme, etc. or into a mRNA which is translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

As used in the present context a nucleic acid molecule may also encompass the untranslated sequence located at the 3' and at the 5' end of the coding gene region, for

example at least 500, preferably 200, especially preferably 100, nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, especially preferably 20, nucleotides of the sequence downstream of the 3' end of the coding gene region. In the event for example the antisense, RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, cosuppression molecule, ribozyme etc. technology is used
 5 coding regions as well as the 5'- and/or 3'-regions can advantageously be used.

However, it is often advantageous only to choose the coding region for cloning and expression purposes.

"Polypeptide" refers to a polymer of amino acid (amino acid sequence) and does not
 10 refer to a specific length of the molecule. Thus, peptides and oligopeptides are included within the definition of polypeptide. This term does also refer to or include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatu-
 15 ral amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

The term "Table I" used in this specification is to be taken to specify the content of Table I A and Table I B. The term "Table II" used in this specification is to be taken to specify the content of Table II A and Table II B. The term "Table I A" used in this specification is to be taken to specify the content of Table I A. The term "Table I B" used in
 20 this specification is to be taken to specify the content of Table I B. The term "Table II A" used in this specification is to be taken to specify the content of Table II A. The term "Table II B" used in this specification is to be taken to specify the content of Table II B. In one preferred embodiment, the term "Table I" means Table I B. In one preferred embodiment, the term "Table II" means Table II B.
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The terms "comprise" or "comprising" and grammatical variations thereof when used in this specification are to be taken to specify the presence of stated features, integers, steps or components or groups thereof, but not to preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

30 **[0053.1.1.1]** In accordance with the invention, a protein or polypeptide has the "activity of an protein as shown in table II, column 3" if its *de novo* activity, or its increased expression directly or indirectly leads to and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof and the protein has the above men-
 35 tioned activities of a protein as shown in table II, column 3. Throughout the specification the activity or preferably the biological activity of such a protein or polypeptide or an nucleic acid molecule or sequence encoding such protein or polypeptide is identical or similar if it still has the biological or enzymatic activity of a protein as shown in table II, column 3, or which has at least 10% of the original biological or enzymatic activity,
 40 preferably 20%, particularly preferably 30%, most particularly preferably 40% in com-

parison to a protein as shown in table II, column 3 of *E. coli* or *Saccharomyces cerevisiae*.

[0054.1.1.1] The terms “increased”, “rised”, “extended”, “enhanced”, “improved” or “amplified” relate to a corresponding change of a property in a plant, an organism, a part of an organism such as a tissue, seed, root, leave, flower etc. or in a cell and are interchangeable. Preferably, the overall activity in the volume is increased or enhanced in cases if the increase or enhancement is related to the increase or enhancement of an activity of a gene product, independent whether the amount of gene product or the specific activity of the gene product or both is increased or enhanced or whether the amount, stability or translation efficacy of the nucleic acid sequence or gene encoding for the gene product is increased or enhanced.

The terms “increase” relate to a corresponding change of a property an organism or in a part of a plant, an organism, such as a tissue, seed, root, leave, flower etc. or in a cell. Preferably, the overall activity in the volume is increased in cases the increase relates to the increase of an activity of a gene product, independent whether the amount of gene product or the specific activity of the gene product or both is increased or generated or whether the amount, stability or translation efficacy of the nucleic acid sequence or gene encoding for the gene product is increased.

Under “change of a property” it is understood that the activity, expression level or amount of a gene product or the metabolite content is changed in a specific volume relative to a corresponding volume of a control, reference or wild type, including the de novo creation of the activity or expression.

The terms “increase” include the change of said property in only parts of the subject of the present invention, for example, the modification can be found in compartment of a cell, like a organelle, or in a part of a plant, like tissue, seed, root, leave, flower etc. but is not detectable if the overall subject, i.e. complete cell or plant, is tested.

Accordingly, the term “increase” means that the specific activity of an enzyme as well as the amount of a compound or metabolite, e.g. of a polypeptide, a nucleic acid molecule of the invention or an encoding mRNA or DNA, can be increased in a volume.

[0055.1.1.1] The terms “wild type”, “control” or “reference” are exchangeable and can be a cell or a part of organisms such as an organelle like a chloroplast or a tissue, or an organism, in particular a plant, which was not modified or treated according to the herein described process according to the invention. Accordingly, the cell or a part of organisms such as an organelle like a chloroplast or a tissue, or an organism, in particular a plant used as wild type, control or reference corresponds to the cell, organism, plant or part thereof as much as possible and is in any other property but in the result of the process of the invention as identical to the subject matter of the invention as possible. Thus, the wild type, control or reference is treated identically or as identical as possible, saying that only conditions or properties might be different which do not influence the quality of the tested property.

Preferably, any comparison is carried out under analogous conditions. The term “analogous conditions” means that all conditions such as, for example, culture or growing conditions, water content of the soil, temperature, humidity or surrounding air or soil, assay conditions (such as buffer composition, temperature, substrates, pathogen strain, concentrations and the like) are kept identical between the experiments to be compared.

The “reference”, “control”, or “wild type” is preferably a subject, e.g. an organelle, a cell, a tissue, an organism, in particular a plant, which was not modified or treated according to the herein described process of the invention and is in any other property as similar to the subject matter of the invention as possible. The reference, control or wild type is in its genome, transcriptome, proteome or metabolome as similar as possible to the subject of the present invention. Preferably, the term “reference-” “control-” or “wild type-”-organelle, -cell, -tissue or -organism, in particular plant, relates to an organelle, cell, tissue or organism, in particular plant, which is nearly genetically identical to the organelle, cell, tissue or organism, in particular plant, of the present invention or a part thereof preferably 95%, more preferred are 98%, even more preferred are 99,00%, in particular 99,10%, 99,30%, 99,50%, 99,70%, 99,90%, 99,99%, 99,999% or more. Most preferable the “reference”, “control”, or “wild type” is a subject, e.g. an organelle, a cell, a tissue, an organism, which is genetically identical to the organism, cell or organelle used according to the process of the invention except that the responsible or activity conferring nucleic acid molecules or the gene product encoded by them are amended, manipulated, exchanged or introduced according to the inventive process.

[0056.1.1.1] In case, a control, reference or wild type differing from the subject of the present invention only by not being subject of the process of the invention can not be provided, a control, reference or wild type can be an organism in which the cause for the modulation of an activity conferring the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof or expression of the nucleic acid molecule of the invention as described herein has been switched back or off, e.g. by knocking out the expression of responsible gene product, e.g. by antisense inhibition, by inactivation of an activator or agonist, by activation of an inhibitor or antagonist, by inhibition through adding inhibitory antibodies, by adding active compounds as e.g. hormones, by introducing negative dominant mutants, etc. A gene production can for example be knocked out by introducing inactivating point mutations, which lead to an enzymatic activity inhibition or a destabilization or an inhibition of the ability to bind to cofactors etc.

[0057.1.1.1] Accordingly, preferred reference subject is the starting subject of the present process of the invention. Preferably, the reference and the subject matter of the invention are compared after standardization and normalization, e.g. to the amount of total RNA, DNA, or Protein or activity or expression of reference genes, like house-keeping genes, such as ubiquitin, actin or ribosomal proteins.

[0058.1.1.1] The increase or modulation according to this invention can be constitutive, e.g. due to a stable permanent transgenic expression or to a stable mutation in the corresponding endogenous gene encoding the nucleic acid molecule of the invention or to a modulation of the expression or of the behavior of a gene conferring the expression of the polypeptide of the invention, or transient, e.g. due to an transient transformation or temporary addition of a modulator such as an agonist or antagonist or inducible, e.g. after transformation with an inducible construct carrying the nucleic acid molecule of the invention under control of an inducible promoter and adding the inducer, e.g. tetracycline or as described herein below.

[0059.1.1.1] The increase in activity of the polypeptide amounts in a cell, a tissue, an organelle, an organ or an organism or a part thereof preferably to at least 5%, preferably to at least 20% or at least 50%, especially preferably to at least 70%, 80%, 90% or more, very especially preferably are to at least 200%, 300% or 400%, most preferably are to at least 500% or more in comparison to the control, reference or wild type.

In one embodiment the term increase means the increase in amount in relation to the weight of the organism or part thereof (w/w).

In one embodiment the increase in activity of the polypeptide amounts in an organelle such as a plastid.

[0060.1.1.1] The specific activity of a polypeptide encoded by a nucleic acid molecule of the present invention or of the polypeptide of the present invention can be tested as described in the examples. In particular, the expression of a protein in question in a cell, e.g. a plant cell in comparison to a control is an easy test and can be performed as described in the state of the art.

[0061.1.1.1] The term "increase" includes, that a compound or an activity is introduced into a cell or a subcellular compartment or organelle *de novo* or that the compound or the activity has not been detectable before, in other words it is "generated".

Accordingly, in the following, the term "increasing" also comprises the term "generating" or "stimulating". The increased activity manifests itself in an increase of the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof.

[0062.1.1.1] The sequence of B0124 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as glucose dehydrogenase.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "glucose dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B0124 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B0124; or
- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B0124 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B0124,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "glucose dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "glucose dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of:

isoleucine, leucine, valine, fructose, homoserine, or any combination of 2, 3, 4, 5, of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a "glucose dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance, abiotic stress resistance and/or content of fine chemical(s).

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "glucose dehydrogenase", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "glucose dehydrogenase", is increased non-targeted.

The sequence of B0730 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published

in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as transcription regulator farR, fatty acyl- responsive.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a “transcription regulator farR, fatty acyl- responsive” from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

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- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B0730 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B0730; or
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- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B0730 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B0730,
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as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

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In one embodiment increasing or generating the activity of a “transcription regulator farR, fatty acyl- responsive.” from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

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In one embodiment increasing or generating the activity of a “transcription regulator farR, fatty acyl- responsive.” from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of:

30 threonine, glutamate, proline, linoleic acid c18:2 (c9,c12), fumaric acid, malic acid, succinic acid, aspartic acid, coenzyme q9, ferulic acid, raffinose, isopentenyl pyrophosphate or any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a “transcription regulator farR, fatty acyl- responsive.” from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in yield, preferably increased biomass.

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In one embodiment increasing or generating the activity of a “transcription regulator farR, fatty acyl- responsive.” from *Escherichia coli* or its functional equivalent or its ho-

molog as mentioned in section (a) or (b) of this paragraph confers an increase in in any combination of the above mentioned traits, e.g. biotic stress resistance, abiotic stress resistance, content of fine chemical(s) and/or increased biomass.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "transcription regulator farR, fatty acyl- responsive", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "transcription regulator farR, fatty acyl- responsive", is increased non-targeted.

The sequence of B1528 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., *Science* 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., *Science* 277 (5331), 1453-1474 (1997), and its activity is published described as chloramphenicol resistance protein homolog ydeA.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "chloramphenicol resistance protein homolog ydeA" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B1528 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B1528; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B1528 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B1528,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "chloramphenicol resistance protein homolog ydeA." from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "chloramphenicol resistance protein homolog ydeA." from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "chloramphenicol resistance protein homolog ydeA", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "chloramphenicol resistance protein homolog ydeA", is increased non-targeted.

The sequence of B2148 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as transmembrane pore-generating protein mglC.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "transmembrane pore-generating protein mglC" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B2148 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B2148; or
- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B2148 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B2148,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "transmembrane pore-generating protein mglC" from *Escherichia coli* or its functional equivalent or its ho-

molog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

5 In one embodiment increasing or generating the activity of a "transmembrane pore-generating protein mglC" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in In one embodiment increasing or generating the activity of a "transmembrane pore-generating protein mglC" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.
10 tance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "transmembrane pore-generating protein mglC", preferably it is the molecule of section (a) or (b) of this paragraph.

15 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "transmembrane pore-generating protein mglC", is increased non-targeted.

The sequence of B2573 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as RNA polymerase sigma-E factor (sigma-24).
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Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "RNA polymerase sigma-E factor (sigma-24)" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of
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- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B2573 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B2573; or
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- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B2573 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B2573,
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as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "RNA polymerase sigma-E factor (sigma-24)", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "RNA polymerase sigma-E factor (sigma-24)", is increased non-targeted.

The sequence of B2600 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., *Science* 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., *Science* 277 (5331), 1453-1474 (1997), and its activity is published described as chorismate mutase-T and prephenate dehydrogenase.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "chorismate mutase-T and prephenate dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B2600 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B2600; or
- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B2600 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B2600,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "chorismate mutase-T and prephenate dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in NUE.

In one embodiment increasing or generating the activity of a "chorismate mutase-T and prephenate dehydrogenase" from *Escherichia coli* or its functional equivalent or its ho-

molog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of: alpha-tocotrienol, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytyl-quinol, gamma-tocotrienol/beta-tocotrienol, tyrosine, shikimic acid, coenzyme q10, alpha-tocopherol, tryptophane or any combination of 2, 3, 4, 5, 6, 7, 8 of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a "chorismate mutase-T and prephenate dehydrogenase" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance, fine chemical content and/or NUE.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "chorismate mutase-T and prephenate dehydrogenase", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "chorismate mutase-T and prephenate dehydrogenase", is increased plastidic.

The sequence of B2699 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as recombinase A.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "recombinase A" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B2699 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B2699; or
- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B2699 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B2699,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

5 In one embodiment increasing or generating the activity of a "recombinase A" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

10 In one embodiment increasing or generating the activity of a "recombinase A" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of: alpha-linolenic acid, alpha-tocopherol, arginine, beta-carotene, beta-sitosterol, campesterol, citrulline, coenzyme q10, coenzyme q9, fumaric acid, glycine, lacton of trihydroxybutyric acid, lutein, methylgalactopyranosid, myo-inositol, proline, raffinose, stearic acid c18:0, trihydroxybutanoic acid, zeaxanthin
15 or any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a "recombinase A" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned
20 traits, e.g. biotic stress resistance, fine chemical content and/or abiotic stress. Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "recombinase A", preferably it is the molecule of section (a) or (b) of this paragraph.

25 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "recombinase A", is increased non-targeted.

The sequence of B2764 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published
30 in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as sulfite reductase (NADPH), flavoprotein beta subunit.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "sulfite reductase (NADPH), flavoprotein beta subunit" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of
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(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B2764 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in

column 7 of Table I B, and being depicted in the same respective line as said B2764; or

- 5 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B2764 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B2764,

10 as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "sulfite reductase (NADPH), flavoprotein beta subunit", preferably it is the molecule of section 15 (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "sulfite reductase (NADPH), flavoprotein beta subunit", is increased non-targeted.

20 The sequence of B3262 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., *Science* 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., *Science* 277 (5331), 1453-1474 (1997), and its activity is published described as b3262-protein.

25 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "b3262-protein" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- 30 (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B3262 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B3262; or

- 35 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B3262 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B3262,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "b3262-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "b3262-protein", is increased non-targeted.

10 The sequence of B3363 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., *Science* 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., *Science* 277 (5331), 1453-1474 (1997), and its activity is published described as peptidyl-prolyl cis-trans isomerase A (rotamase A).

15 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "peptidyl-prolyl cis-trans isomerase A (rotamase A)" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

20 (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B3363 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B3363; or

25 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B3363 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same
30 respective line as said B3363,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

35 In one embodiment increasing or generating the activity of a "peptidyl-prolyl cis-trans isomerase A (rotamase A)" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content and increased yield, preferably increased biomass.

In one embodiment increasing or generating the activity of a "peptidyl-prolyl cis-trans isomerase A (rotamase A)" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance, increased biomass and/or abiotic stress. Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "peptidyl-prolyl cis-trans isomerase A (rotamase A)", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "peptidyl-prolyl cis-trans isomerase A (rotamase A)", is increased non-targeted.

The sequence of B3644 from *Escherichia coli*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as b3644-protein.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "b3644-protein" from *Escherichia coli* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said B3644 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said B3644; or
- (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said B3644 or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said B3644,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "b3644-protein" from *Escherichia coli* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of: α -linolenic acid (c18:cis [9,12,15]3), cerotic acid

(c26:0), coenzyme q9, fumarate, glycerol, lignoceric acid (c24:0), linoleic acid (c18:cis [9,12]2), palmitic acid (c16:0), praline or any combination of 2, 3, 4, 5, 6, 7, 8, 9 of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a "b3644-protein" from Escherichia coli or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or fine chemical content.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "b3644-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "b3644-protein", is increased non-targeted.

The sequence of YCR087W from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YCR087W-protein.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YCR087W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YCR087W or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YCR087W; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YCR087W or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YCR087W,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "YCR087W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in

section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "YCR087W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in
 5 section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "YCR087W-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

10 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YCR087W-protein", is increased non-targeted.

The sequence of YGR150C from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has
 15 been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YGR150c-protein.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YGR150c-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g.
 20 the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YGR150C or a functional equivalent or a homologue thereof as shown depicted in column 7
 25 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YGR150C; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same
 30 respective line as said YGR150C or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YGR150C,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant
 35 cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "YGR150c-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in

section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "YGR150c-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in
 5 section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "YGR150c-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

10 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YGR150c-protein", is increased non-targeted.

The sequence of YIL172C from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau
 15 et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YIL172C-protein.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YIL172C-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g.
 20 the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YIL172C or a functional equivalent or a homologue thereof as shown depicted in column 7
 25 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YIL172C; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same
 30 respective line as said YIL172C or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YIL172C,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant
 35 cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "YIL172C-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in

section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "YIL172C-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in
 5 section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "YIL172C-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

10 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YIL172C-protein", is increased non-targeted.

The sequence of YLR168C from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau
 15 et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YLR168C-protein.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YLR168C-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g.
 20 the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YLR168C or a functional equivalent or a homologue thereof as shown depicted in column 7
 25 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YLR168C; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same
 30 respective line as said YLR168C or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YLR168C,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant
 35 cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "YLR168C-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YLR168C-protein", is increased non-targeted.

5 The sequence of YLR407W from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YLR407W-protein.

10 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YLR407W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

15 (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YLR407W or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YLR407W; or

20 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YLR407W or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YLR407W,

25 as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

30 In one embodiment increasing or generating the activity of a "YLR407W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

35 In one embodiment increasing or generating the activity of a "YLR407W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "YLR407W-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YLR407W-protein", is increased non-targeted.

5 The sequence of YNL241C from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as glucose-6-phosphate 1-dehydrogenase.

10 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "glucose-6-phosphate 1-dehydrogenase" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

15 (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YNL241C or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YNL241C; or

20 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YNL241C or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YNL241C,

25 as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

30 In one embodiment increasing or generating the activity of a "glucose-6-phosphate 1-dehydrogenase" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in NUE.

In one embodiment increasing or generating the activity of a "glucose-6-phosphate 1-dehydrogenase" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in content of at least one fine chemical selected from the group consisting of: a-linolenic acid, 35 c18:3 (c9,c12,c15), alanine, arginine, campesterol, fructose, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose, glyceric acid, hexadeca-dienoic acid (c16:cis [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), isoleucine, succinate, threonine, tryptophane, tyrosine, valine or any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 of the above mentioned fine chemicals.

In one embodiment increasing or generating the activity of a "glucose-6-phosphate 1-dehydrogenase" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in NUE.

5 In one embodiment increasing or generating the activity of a "glucose-6-phosphate 1-dehydrogenase" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance, fine chemical content and/or NUE.

10 Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "glucose-6-phosphate 1-dehydrogenase", preferably it is the molecule of section (a) or (b) of this paragraph.

15 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "glucose-6-phosphate 1-dehydrogenase", is increased plastidic.

20 The sequence of YOR260W from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as Gamma subunit of the translation initiation factor eIF2B.

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "Gamma subunit of the translation initiation factor eIF2B" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

25 (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YOR260W or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YOR260W; or

30 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YOR260W or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YOR260W,

35 as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "Gamma subunit of the translation initiation factor eIF2B" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

- 5 In one embodiment increasing or generating the activity of a "Gamma subunit of the translation initiation factor eIF2B" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.
- 10 Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "Gamma subunit of the translation initiation factor eIF2B", preferably it is the molecule of section (a) or (b) of this paragraph.

- 15 In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "Gamma subunit of the translation initiation factor eIF2B", is increased non-targeted.

- 20 The sequence of YOR299W from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as YOR299W-protein.

- 25 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "YOR299W-protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

- (a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YOR299W or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YOR299W; or
- 30 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YOR299W or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YOR299W,
- 35

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

Accordingly, in one embodiment, the molecule which activity is to be increased in the
 5 process of the invention is the gene product with an activity of described as a "YOR299W-protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "YOR299W-protein", is increased non-targeted.

10 The sequence of YPL079W from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as ribosomal protein.

15 Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "ribosomal protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YPL079W
 20 or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YPL079W; or

25 (b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YPL079W or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the
 30 same respective line as said YPL079W,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "ribosomal protein" from
 35 *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "ribosomal protein" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in

section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "ribosomal protein", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "ribosomal protein", is increased non-targeted.

The sequence of YPL151C from *Saccharomyces cerevisiae*, e.g. as shown in column 5 of Table I, [sequences from *Saccharomyces cerevisiae* has been published in Goffeau et al., Science 274 (5287), 546-547, 1996, sequences from *Escherichia coli* has been published in Blattner et al., Science 277 (5331), 1453-1474 (1997), and its activity is published described as Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p).

Accordingly, in one embodiment, the process of the present invention comprises increasing or generating the activity of a gene product with the activity of a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog, e.g. the increase of

(a) a gene product of a gene comprising the nucleic acid molecule as shown in column 5 of Table I and being depicted in the same respective line as said YPL151C or a functional equivalent or a homologue thereof as shown depicted in column 7 of Table I, preferably a homologue or functional equivalent as shown depicted in column 7 of Table I B, and being depicted in the same respective line as said YPL151C; or

(b) a polypeptide comprising a polypeptide, a consensus sequence or a polypeptide motif as shown depicted in column 5 of Table II, and being depicted in the same respective line as said YPL151C or a functional equivalent or a homologue thereof as depicted in column 7 of Table II or IV, preferably a homologue or functional equivalent as depicted in column 7 of Table II B, and being depicted in the same respective line as said YPL151C,

as mentioned herein, for the an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof in plant cell, plant or part thereof, as mentioned.

In one embodiment increasing or generating the activity of a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in abiotic stress, preferably drought and low water content.

In one embodiment increasing or generating the activity of a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" from *Saccharomyces cerevisiae* or its functional equivalent or its homolog as mentioned in section (a) or (b) of this paragraph confers an increase in any combination of the above mentioned traits, e.g. biotic stress resistance and/or abiotic stress resistance.

Accordingly, in one embodiment, the molecule which activity is to be increased in the process of the invention is the gene product with an activity of described as a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)", preferably it is the molecule of section (a) or (b) of this paragraph.

In one embodiment, said molecule, which activity is to be increased in the process of the invention and which is the gene product with an activity of described as a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)", is increased non-targeted.

[0063.1.1.1] Surprisingly, it was observed that a increasing or generating of at least one gene conferring an activity selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein or of a gene comprising a nucleic acid sequence described in column 5 of Table I in *Arabidopsis thaliana*, preferably eco-type C24 conferred an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control .

[0064.1.1.1] Surprisingly, it was observed that a increasing or generating of at least one gene conferring an activity selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein or of a gene comprising a nucleic acid sequence described in column 5 of Table I in *Arabidopsis thaliana*, preferably eco-type C24 conferred an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "glucose dehydrogenase" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 38 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

5 It was further observed that increasing or generating the activity of a gene product with the activity of a "glucose dehydrogenase" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 38 in *Arabidopsis thaliana* conferred additionally an increase

10 in abiotic stress resistance, preferably drought and low water content and in content of at least one fine chemical selected from the group consisting of: isoleucine, leucine, valine, fructose, homoserine, isoleucine.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "transcription regulator farR, fatty acyl- responsive" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 146 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

15 It was further observed that increasing or generating the activity of a gene product with the activity of a "transcription regulator farR, fatty acyl- responsive" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 146 in *Arabidopsis thaliana* conferred additionally an increase

20 in abiotic stress resistance, preferably drought and low water content and yield, preferably increased biomass and in content of at least one fine chemical selected from the group consisting of: threonine, glutamate, proline, linoleic acid c18:2 (c9,c12), fumaric acid, malic acid, succinic acid, aspartic acid, coenzyme q9, ferulic acid, raffinose, isopentenyl pyrophosphate.

25 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "chloramphenicol resistance protein homolog ydeA" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 165 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

30 It was further observed that increasing or generating the activity of a gene product with the activity of a "chloramphenicol resistance protein homolog ydeA" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 165 in *Arabidopsis thaliana* conferred additionally an increase

35 in abiotic stress resistance, preferably drought and low water content.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "transmembrane pore-generating protein mglC" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 242 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

40

It was further observed that increasing or generating the activity of a gene product with the activity of a “transmembrane pore-generating protein mglC” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 242 in *Arabidopsis thaliana* conferred additionally an increase

5 in abiotic stress resistance, preferably drought and low water content.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “RNA polymerase sigma-E factor (sigma-24)” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 312 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as

10 shown in the Examples.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “chorismate mutase-T and prephenate dehydrogenase” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 468 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

15 It was further observed that increasing or generating the activity of a gene product with the activity of a “chorismate mutase-T and prephenate dehydrogenase” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 468 in *Arabidopsis thaliana* conferred additionally an increase

20 in NUE and in content of at least one fine chemical selected from the group consisting of: alpha-tocotrienol, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytyl-quinol, gamma-tocotrienol/beta-tocotrienol, tyrosine, shikimic acid, coenzyme q10, alpha-tocopherol, tryptophane

25 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “recombinase A” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 534 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

30 It was further observed that increasing or generating the activity of a gene product with the activity of a “recombinase A” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 534 in *Arabidopsis thaliana* conferred an increase

35 in abiotic stress resistance, preferably drought and low water content and in content of at least one fine chemical selected from the group consisting of: alpha-linolenic acid, alpha-tocopherol, arginine, beta-carotene, beta-sitosterol, campesterol, citrulline, coenzyme q10, coenzyme q9, fumaric acid, glycine, lacton of trihydroxybutyric acid, lutein, methylgalactopyranosid, myo-inositol, proline, raffinose, stearic acid c18:0, trihydroxybutanoic acid, zeaxanthin

40 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “sulfite reductase (NADPH), flavoprotein beta subunit” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 970 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi

as shown in the Examples.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “b3262-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1143 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “peptidyl-prolyl cis-trans isomerase A (rotamase A)” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1174 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

It was further observed that increasing or generating the activity of a gene product with the activity of a “peptidyl-prolyl cis-trans isomerase A (rotamase A)” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1174 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content and increased yield, preferably increased biomass.

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “b3644-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1521 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

It was further observed that increasing or generating the activity of a gene product with the activity of a “b3644-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1521 in *Arabidopsis thaliana* conferred additionally an increase in content of at least one fine chemical selected from the group consisting of: α -linolenic acid (c18:cis [9,12,15]3), cerotic acid (c26:0), coenzyme q9, fumarate, glycerol, lignoceric acid (c24:0), linoleic acid (c18:cis [9,12]2), palmitic acid (c16:0), proline

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “YCR087W-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1660 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

It was further observed that increasing or generating the activity of a gene product with the activity of a “YCR087W-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1660 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content

In particular, it was observed that increasing or generating the activity of a gene product with the activity of a “YGR150c-protein” encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1664 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

It was further observed that increasing or generating the activity of a gene product with the activity of a "YGR150c-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1664 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content

- 5 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "YIL172C-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1691 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

- 10 It was further observed that increasing or generating the activity of a gene product with the activity of a "YIL172C-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1691 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content

- 15 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "YLR168C-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1859 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

- 20 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "YLR407W-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1904 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

- 25 It was further observed that increasing or generating the activity of a gene product with the activity of a "YLR407W-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1904 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content

- 30 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "glucose-6-phosphate 1-dehydrogenase" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1920 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

- It was further observed that increasing or generating the activity of a gene product with the activity of a "glucose-6-phosphate 1-dehydrogenase" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 1920 in *Arabidopsis thaliana* conferred additionally an increase

- 35 in NUE and in content of at least one fine chemical selected from the group consisting of: a-linolenic acid, c18:3 (c9,c12,c15), alanine, arginine, campesterol, fructose, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose, glyceric acid, hexadeca-dienoic acid (c16:cis [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), isoleucine, succinate, threonine, tryptophane, tyrosine, valine
40 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "Gamma subunit of the translation initiation factor eIF2B" encoded by a gene compris-

ing the nucleic acid sequence SEQ ID NO.: 2455 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

5 It was further observed that increasing or generating the activity of a gene product with the activity of a "Gamma subunit of the translation initiation factor eIF2B" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2455 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress, resistance, preferably drought and low water content

10 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "YOR299W-protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2477 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

15 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "ribosomal protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2507 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

It was further observed that increasing or generating the activity of a gene product with the activity of a "ribosomal protein" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2507 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content

20 In particular, it was observed that increasing or generating the activity of a gene product with the activity of a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2683 in *Arabidopsis thaliana* conferred an increased resistance to biotic stress, preferably pathogenic fungi as shown in the Examples.

25 It was further observed that increasing or generating the activity of a gene product with the activity of a "Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p)" encoded by a gene comprising the nucleic acid sequence SEQ ID NO.: 2683 in *Arabidopsis thaliana* conferred additionally an increase in abiotic stress resistance, preferably drought and low water content.

[0065.1.1.1] Thus, according to the method of the invention an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control can be achieved.

35 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 39, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 38 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in
40 Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ

ID NO.: 38 or polypeptide SEQ ID NO.: 39, respectively is increased or generated or if the activity "glucose dehydrogenase" is increased or generated in an organism, preferably

5 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 147, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 146 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 146 or polypeptide SEQ ID NO.: 147, respectively is increased or generated or if the activity "transcription regulator farR, fatty acyl- responsive" is increased or generated in an organism, preferably

15 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 166, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 165 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 165 or polypeptide SEQ ID NO.: 166, respectively is increased or generated or if the activity "chloramphenicol resistance protein homolog ydeA" is increased or generated in an organism, preferably

25 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 243, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 242 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 242 or polypeptide SEQ ID NO.: 243, respectively is increased or generated or if the activity "transmembrane pore-generating protein mglC" is increased or generated in an organism, preferably

35 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 313, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 312 or a homolog of said nucleic acid molecule or polypep-

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5 tide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 312 or polypeptide SEQ ID NO.: 313, respectively is increased or generated or if the activity "RNA polymerase sigma-E factor (sigma-24)" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

10 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 469, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 468 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 468 or polypeptide SEQ ID NO.: 469, respectively is increased or generated or if the activity "chorismate mutase-T and prephenate dehydrogenase" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

20 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 535, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 534 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 534 or polypeptide SEQ ID NO.: 535, respectively is increased or generated or if the activity "recombinase A" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

35 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 971, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 970 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 970 or polypeptide SEQ ID NO.: 971, respectively is increased or generated or if the activity "sulfite reductase (NADPH), flavoprotein beta subunit" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1144, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1143 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1143 or polypeptide SEQ ID NO.: 1144, respectively is increased or generated or if the activity "b3262-protein" is increased or generated in an organism, preferably

increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1175, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1174 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1174 or polypeptide SEQ ID NO.: 1175, respectively is increased or generated or if the activity "peptidyl-prolyl cis-trans isomerase A (rotamase A)" is increased or generated in an organism, preferably

increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1522, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1521 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1521 or polypeptide SEQ ID NO.: 1522, respectively is increased or generated or if the activity "b3644-protein" is increased or generated in an organism, preferably

increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1661, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1660 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1660 or polypeptide SEQ ID NO.: 1661, respectively is increased or generated or if the activity "YCR087W-protein" is increased or generated in

an organism, preferably

increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

5 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1665, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1664 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid
10 molecule SEQ ID NO.: 1664 or polypeptide SEQ ID NO.: 1665, respectively is increased or generated or if the activity "YGR150c-protein" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

15 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1692, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1691 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid
20 molecule SEQ ID NO.: 1691 or polypeptide SEQ ID NO.: 1692, respectively is increased or generated or if the activity "YIL172C-protein" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a
25 corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1860, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1859 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid
30 molecule SEQ ID NO.: 1859 or polypeptide SEQ ID NO.: 1860, respectively is increased or generated or if the activity "YLR168C-protein" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a
35 corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1905, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1904 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as de-
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picted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1904 or polypeptide SEQ ID NO.: 1905, respectively is increased or generated or if the activity "YLR407W-protein" is increased or generated in an organism, preferably

- 5 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 1921, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 1920 or a homolog of said nucleic acid molecule or polypep-
10 tide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 1920 or polypeptide SEQ ID NO.: 1921, respectively is in-
15 creased or generated or if the activity "glucose-6-phosphate 1-dehydrogenase" is increased or generated in an organism, preferably
increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 2456, or encoded by a nucleic acid molecule comprising the
20 nucleic acid SEQ ID NO.: 2455 or a homolog of said nucleic acid molecule or polypep- tide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2455 or polypeptide SEQ ID NO.: 2456, respectively is in-
25 creased or generated or if the activity "Gamma subunit of the translation initiation factor eIF2B" is increased or generated in an organism, preferably
increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 2478, or encoded by a nucleic acid molecule comprising the
30 nucleic acid SEQ ID NO.: 2477 or a homolog of said nucleic acid molecule or polypep- tide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid
35 molecule SEQ ID NO.: 2477 or polypeptide SEQ ID NO.: 2478, respectively is in- creased or generated or if the activity "YOR299W-protein" is increased or generated in an organism, preferably
increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.

40 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 2508, or encoded by a nucleic acid molecule comprising the

- nucleic acid SEQ ID NO.: 2507 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2507 or polypeptide SEQ ID NO.: 2508, respectively is increased or generated or if the activity "ribosomal protein" is increased or generated in an organism, preferably increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.
- 10 Accordingly, in one embodiment, in case the activity of a polypeptide according to the polypeptide SEQ ID NO.: 2684, or encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO.: 2683 or a homolog of said nucleic acid molecule or polypeptide, e.g. if the activity of a nucleic acid molecule or a polypeptide comprising the nucleic acid or polypeptide or the consensus sequence or the polypeptide motif, as depicted in Table I, II or IV, column 7 in the respective same line as the nucleic acid molecule SEQ ID NO.: 2683 or polypeptide SEQ ID NO.: 2684, respectively is increased or generated or if the activity "Splicing factor that is found in the Cef1p sub-complex of the spliceosome (Prp46p)" is increased or generated in an organism, preferably
- 15 increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control is conferred in said organism.
- 20

[0066.1.1.1] The term "expression" refers to the transcription and/or translation of a codogenic gene segment or gene. As a rule, the resulting product is an mRNA or a protein. However, expression products can also include functional RNAs such as, for example, antisense, nucleic acids, tRNAs, snRNAs, rRNAs, RNAi, siRNA, ribozymes etc. Expression may be systemic, local or temporal, for example limited to certain cell types, tissues organs or organelles or time periods.

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[0067.1.1.1] In one embodiment, the process of the present invention comprises one or more of the following steps

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- a) stabilizing a protein conferring the increased expression of a protein encoded by the nucleic acid molecule of the invention or of the polypeptide of the invention having the herein-mentioned activity selected from the group consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase
- 35 (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive,
- 40

transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof ;

b) stabilizing a mRNA conferring the increased expression of a protein encoded by the nucleic acid molecule of the invention or its homologs or of a mRNA encoding the polypeptide of the present invention having the herein-mentioned activity selected from the group consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control;

c) increasing the specific activity of a protein conferring the increased expression of a protein encoded by the nucleic acid molecule of the invention or of the polypeptide of the present invention or decreasing the inhibitory regulation of the polypeptide of the invention;

d) generating or increasing the expression of an endogenous or artificial transcription factor mediating the expression of a protein conferring the increased expression of a protein encoded by the nucleic acid molecule of the invention or of the polypeptide of the invention having the herein-mentioned activity selected from the group consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control;

e) stimulating activity of a protein conferring the increased expression of a protein encoded by the nucleic acid molecule of the present invention or a polypeptide of the present invention having the herein-mentioned activity selected from the group

consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control;

f) expressing a transgenic gene encoding a protein conferring the increased expression of a polypeptide encoded by the nucleic acid molecule of the present invention or a polypeptide of the present invention, having the herein-mentioned activity selected from the group consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control; and/or

g) increasing the copy number of a gene conferring the increased expression of a nucleic acid molecule encoding a polypeptide encoded by the nucleic acid molecule of the invention or the polypeptide of the invention having the herein-mentioned activity selected from the group consisting of b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein and conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control;

- h) increasing the expression of the endogenous gene encoding the polypeptide of the invention or its homologs by adding positive expression or removing negative expression elements, e.g. homologous recombination can be used to either introduce positive regulatory elements like for plants the 35S enhancer into the promoter or to remove repressor elements from regulatory regions. Further gene conversion methods can be used to disrupt repressor elements or to enhance to activity of positive elements- positive elements can be randomly introduced in plants by T-DNA or transposon mutagenesis and lines can be identified in which the positive elements have been integrated near to a gene of the invention, the expression of which is thereby enhanced;
and/or
- i) modulating growth conditions of the plant in such a manner, that the expression or activity of the gene encoding the protein of the invention or the protein itself is enhanced;
- j) selecting of organisms with especially high activity of the proteins of the invention from natural or from mutagenized resources and breeding them into the target organisms, e.g. the elite crops.

[0068.1.1.1] Preferably, said mRNA is the nucleic acid molecule of the present invention and/or the protein conferring the increased expression of a protein encoded by the nucleic acid molecule of the present invention alone or linked to a transit nucleic acid sequence or transit peptide encoding nucleic acid sequence or the polypeptide having the herein mentioned activity, e.g. conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof after increasing the expression or activity of the encoded polypeptide or having the activity of a polypeptide having an activity as the protein as shown in table II column 3 or its homologs.

[0069.1.1.1] In general, the amount of mRNA or polypeptide in a cell or a compartment of an organism correlates with the amount of encoded protein and thus with the overall activity of the encoded protein in said volume. Said correlation is not always linear, the activity in the volume is dependent on the stability of the molecules or the presence of activating or inhibiting co-factors. Further, product and educt inhibitions of enzymes are well known and described in textbooks, e.g. Stryer, Biochemistry.

[0070.1.1.1] In general, the amount of mRNA, polynucleotide or nucleic acid molecule in a cell or a compartment of an organism correlates with the amount of encoded protein and thus with the overall activity of the encoded protein in said volume. Said correlation is not always linear, the activity in the volume is dependent on the stability of the molecules, the degradation of the molecules or the presence of activating or inhibiting co-factors. Further, product and educt inhibitions of enzymes are well known, e.g. Zinser et al. "Enzyminhibitoren"/Enzyme inhibitors".

- [0071.1.1.1]** The activity of the abovementioned proteins and/or polypeptides encoded by the nucleic acid molecule of the present invention can be increased in various ways. For example, the activity in an organism or in a part thereof, like a cell, is increased via increasing the gene product number, e.g. by increasing the expression rate, like introducing a stronger promoter, or by increasing the stability of the mRNA expressed, thus increasing the translation rate, and/or increasing the stability of the gene product, thus reducing the proteins decayed. Further, the activity or turnover of enzymes can be influenced in such a way that a reduction or increase of the reaction rate or a modification (reduction or increase) of the affinity to the substrate results, is reached. A mutation in the catalytic center of an polypeptide of the invention, e.g. as enzyme, can modulate the turn over rate of the enzyme, e.g. a knock out of an essential amino acid can lead to a reduced or completely knock out activity of the enzyme, or the deletion or mutation of regulator binding sites can reduce a negative regulation like a feedback inhibition (or a substrate inhibition, if the substrate level is also increased).
- [0072.1.1.1]** Moreover, the regulation of the abovementioned nucleic acid sequences may be modified so that gene expression is increased. This can be achieved advantageously by means of heterologous regulatory sequences or by modifying, for example mutating, the natural regulatory sequences which are present. The advantageous methods may also be combined with each other.
- [0073.1.1.1]** In general, an activity of a gene product in an organism or part thereof, in particular in a plant cell or organelle of a plant cell, a plant, or a plant tissue or a part thereof or in a microorganism can be increased by increasing the amount of the specific encoding mRNA or the corresponding protein in said organism or part thereof. "Amount of protein or mRNA" is understood as meaning the molecule number of polypeptides or mRNA molecules in an organism, a tissue, a cell or a cell compartment. "Increase" in the amount of a protein means the quantitative increase of the molecule number of said protein in an organism, a tissue, a cell or a cell compartment such as an organelle like a plastid or mitochondria or part thereof - for example by one of the methods described herein below - in comparison to a wild type, control or reference.
- [0074.1.1.1]** The increase in molecule number amounts preferably to at least 1%, preferably to more than 10%, more preferably to 30% or more, especially preferably to 50%, 70% or more, very especially preferably to 100%, most preferably to 500% or more. However, a de novo expression is also regarded as subject of the present invention.
- [0075.1.1.1]** A modification, i.e. an increase, can be caused by endogenous or exogenous factors. For example, an increase in activity in an organism or a part thereof

can be caused by adding a gene product or a precursor or an activator or an agonist to the media or nutrition or can be caused by introducing said subjects into a organism, transient or stable. Furthermore such an increase can be reached by the introduction of the inventive nucleic acid sequence or the encoded protein in the correct cell compartment for example into the nucleus, or cytoplasm respectively or into plastids either by transformation and/or targeting.

[0076.1.1.1] In one embodiment the increase or decrease in tolerance and/or resistance to environmental stress as compared to a corresponding non-transformed wild type plant cell in the plant or a part thereof, e.g. in a cell, a tissue, a organ, an organelle etc., is achieved by increasing the endogenous level of the polypeptide of the invention. Accordingly, in an embodiment of the present invention, the present invention relates to a process wherein the gene copy number of a gene encoding the polynucleotide or nucleic acid molecule of the invention is increased. Further, the endogenous level of the polypeptide of the invention can for example be increased by modifying the transcriptional or translational regulation of the polypeptide.

[0077.1.1.1] In one embodiment the increased tolerance and/or resistance to environmental stress in the plant or part thereof can be altered by targeted or random mutagenesis of the endogenous genes of the invention. For example homologous recombination can be used to either introduce positive regulatory elements like for plants the 35S enhancer into the promoter or to remove repressor elements form regulatory regions. In addition gene conversion like methods described by Kochevenko and Willmitzer (Plant Physiol. 2003 May;132(1):174-84) and citations therein can be used to disrupt repressor elements or to enhance to activity of positive regulatory elements. Furthermore positive elements can be randomly introduced in (plant) genomes by T-DNA or transposon mutagenesis and lines can be screened for, in which the positive elements has be integrated near to a gene of the invention, the expression of which is thereby enhanced. The activation of plant genes by random integrations of enhancer elements has been described by Hayashi et al., 1992 (Science 258:1350-1353) or Weigel et al., 2000 (Plant Physiol. 122, 1003-1013) and others cited therein. Reverse genetic strategies to identify insertions (which eventually carrying the activation elements) near in genes of interest have been described for various cases e.g.. Krysan et al., 1999 (Plant Cell 1999, 11, 2283-2290); Sessions et al., 2002 (Plant Cell 2002, 14, 2985-2994); Young et al., 2001, (Plant Physiol. 2001, 125, 513-518); Koprek et al., 2000 (Plant J. 2000, 24, 253-263) ; Jeon et al., 2000 (Plant J. 2000, 22, 561-570) ; Tissier et al., 1999 (Plant Cell 1999, 11, 1841-1852); Speulmann et al., 1999 (Plant Cell 1999 ,11 , 1853-1866). Briefly material from all plants of a large T-DNA or transposon mutagenized plant population is harvested and genomic DNA prepared. Then the genomic DNA is pooled following specific architectures as described for example in Krysan et al., 1999 (Plant Cell 1999, 11, 2283-2290). Pools of genomics DNAs are then screened by specific multiplex PCR reactions detecting the combination of the insertional mutagen (eg T-DNA or Transposon) and the gene of interest. There-

fore PCR reactions are run on the DNA pools with specific combinations of T-DNA or transposon border primers and gene specific primers. General rules for primer design can again be taken from Krysan et al., 1999 (Plant Cell 1999, 11, 2283-2290) Re-screening of lower levels DNA pools lead to the identification of individual plants in which the gene of interest is activated by the insertional mutagen.

The enhancement of positive regulatory elements or the disruption or weakening of negative regulatory elements can also be achieved through common mutagenesis techniques: The production of chemically or radiation mutated populations is a common technique and known to the skilled worker. Methods for plants are described by Koorn-eef et al. 1982 and the citations therein and by Lightner and Caspar in "Methods in Molecular Biology" Vol 82. These techniques usually induce pointmutations that can be identified in any known gene using methods such as TILLING (Colbert et al. 2001).

[0078.1.1.1] Accordingly, the expression level can be increased if the endogenous genes encoding a polypeptide conferring an increased expression of the polypeptide of the present invention, in particular genes comprising the nucleic acid molecule of the present invention, are modified via homologous recombination, Tilling approaches or gene conversion. It also possible to add as mentioned herein targeting sequences to the inventive nucleic acid sequences.

[0079.1.1.1] Regulatory sequences preferably in addition to a target sequence or part thereof can be operatively linked to the coding region of an endogenous protein and control its transcription and translation or the stability or decay of the encoding mRNA or the expressed protein. In order to modify and control the expression, promoter, UTRs, splicing sites, processing signals, polyadenylation sites, terminators, enhancers, repressors, post transcriptional or posttranslational modification sites can be changed, added or amended. For example, the activation of plant genes by random integrations of enhancer elements has been described by Hayashi et al., 1992 (Science 258:1350-1353) or Weigel et al., 2000 (Plant Physiol. 122, 1003-1013) and others cited therein. For example, the expression level of the endogenous protein can be modulated by replacing the endogenous promoter with a stronger transgenic promoter or by replacing the endogenous 3'UTR with a 3'UTR, which provides more stability without amending the coding region. Further, the transcriptional regulation can be modulated by introduction of an artificial transcription factor as described in the examples. Alternative promoters, terminators and UTR are described below.

[0080.1.1.1] The activation of an endogenous polypeptide having above-mentioned activity, e.g. having the activity of a protein as shown in table II, column 3 or of the polypeptide of the invention, e.g. conferring the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof after increase of expression or activity in the cytosol and/or in an organelle like a plastid, can also be increased by introducing a synthetic transcription factor, which binds close to the coding region of the gene encoding the protein as shown in table II, column 3 and activates its transcription. A chimeric

zinc finger protein can be constructed, which comprises a specific DNA-binding domain and an activation domain as e.g. the VP16 domain of Herpes Simplex virus. The specific binding domain can bind to the regulatory region of the gene encoding the protein as shown in table II, column 3. The expression of the chimeric transcription factor in a
5 organism, in particular in a plant, leads to a specific expression of the protein as shown in table II, column 3, see e.g. in WO01/52620, Oriz, Proc. Natl. Acad. Sci. USA, 2002, Vol. 99, 13290 or Guan, Proc. Natl. Acad. Sci. USA, 2002, Vol. 99, 13296.

[0081.1.1.1] In one further embodiment of the process according to the invention, organisms are used in which one of the abovementioned genes, or one of the above-
10 mentioned nucleic acids, is mutated in a way that the activity of the encoded gene products is less influenced by cellular factors, or not at all, in comparison with the un-mutated proteins. For example, well known regulation mechanism of enzymic activity are substrate inhibition or feed back regulation mechanisms. Ways and techniques for the introduction of substitution, deletions and additions of one or more bases, nucleotides or amino acids of a corresponding sequence are described herein below in the
15 corresponding paragraphs and the references listed there, e.g. in Sambrook et al., Molecular Cloning, Cold Spring Harbour, NY, 1989. The person skilled in the art will be able to identify regulation domains and binding sites of regulators by comparing the sequence of the nucleic acid molecule of the present invention or the expression product thereof with the state of the art by computer software means which comprise algo-
20 rithms for the identifying of binding sites and regulation domains or by introducing into a nucleic acid molecule or in a protein systematically mutations and assaying for those mutations which will lead to an increased specific activity or an increased activity per volume, in particular per cell.

[0082.1.1.1] It can therefore be advantageous to express in an organism a nucleic acid molecule of the invention or a polypeptide of the invention derived from a evolutionary distantly related organism, as e.g. using a prokaryotic gene in a eukaryotic host, as in these cases the regulation mechanism of the host cell may not weaken the activity (cellular or specific) of the gene or its expression product.

[0083.1.1.1] The mutation is introduced in such a way that the increased yield under condition of transient and repetitive abiotic stress is not adversely affected.

[0084.1.1.1] Less influence on the regulation of a gene or its gene product is understood as meaning a reduced regulation of the enzymatic activity leading to an increased specific or cellular activity of the gene or its product. An increase of the enzymatic activity is understood as meaning an enzymatic activity, which is increased by at
35 least 10%, advantageously at least 20, 30 or 40%, especially advantageously by at least 50, 60 or 70% in comparison with the starting organism. This leads to an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof .

[0085.1.1.1] The invention provides that the above methods can be performed such that the stress tolerance is increased. It is also possible to obtain a decrease in stress tolerance.

5 **[0086.1.1.1]** The invention is not limited to specific nucleic acids, specific polypeptides, specific cell types, specific host cells, specific conditions or specific methods etc. as such, but may vary and numerous modifications and variations therein will be apparent to those skilled in the art. It is also to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

10 **[0087.1.1.1]** The present invention also relates to isolated nucleic acids comprising a nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule encoding the polypeptide shown in column 7 of Table II B;
- b) a nucleic acid molecule shown in column 7 of Table I B;
- 15 c) a nucleic acid molecule, which, as a result of the degeneracy of the genetic code, can be derived from a polypeptide sequence depicted in column 5 or 7 of Table II and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
- 20 d) a nucleic acid molecule having at least 30 % identity with the nucleic acid molecule sequence of a polynucleotide comprising the nucleic acid molecule shown in column 5 or 7 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
- 25 e) a nucleic acid molecule encoding a polypeptide having at least 30 % identity with the amino acid sequence of the polypeptide encoded by the nucleic acid molecule of (a) to (c) and having the activity represented by a nucleic acid molecule comprising a polynucleotide as depicted in column 5
- 30 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- f) nucleic acid molecule which hybridizes with a nucleic acid molecule of (a) to (c) under stringent hybridization conditions and confers an increased
- 35 resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- g) a nucleic acid molecule encoding a polypeptide which can be isolated with the aid of monoclonal or polyclonal antibodies made against a polypeptide

encoded by one of the nucleic acid molecules of (a) to (e) and having the activity represented by the nucleic acid molecule comprising a polynucleotide as depicted in column 5 of Table I;

- 5 h) a nucleic acid molecule encoding a polypeptide comprising the consensus sequence or one or more polypeptide motifs as shown in column 7 of Table IV and preferably having the activity represented polypeptide comprising a polypeptide as depicted in column 5 of Table II or IV;
- 10 h) a nucleic acid molecule encoding a polypeptide having the activity represented by a protein as depicted in column 5 of Table II and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 15 i) nucleic acid molecule which comprises a polynucleotide, which is obtained by amplifying a cDNA library or a genomic library using the primers in column 7 of Table III and preferably having the activity represented by a polypeptide comprising a polypeptide as depicted in column 5 of Table II or IV;
- and
- 20 j) a nucleic acid molecule which is obtainable by screening a suitable nucleic acid library under stringent hybridization conditions with a probe comprising a complementary sequence of a nucleic acid molecule of (a) or (b) or with a fragment thereof, having at least 15 nt, preferably 20 nt, 30 nt, 50 nt, 100 nt, 200 nt or 500 nt of a nucleic acid molecule complementary to a nucleic acid molecule sequence characterized in (a) to (e) and encoding a polypeptide having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II;
- 25

whereby the nucleic acid molecule according to (a) to (j) is at least in one or more nucleotides different from the sequence depicted in column 5 or 7 of Table I A and preferably which encodes a protein which differs at least in one or more amino acids from the protein sequences depicted in column 5 or 7 of Table II A.

[0088.1.1.1] In one embodiment, the homolog of the any one of the polypeptides indicated in Table II, column 3, is a homolog having the same or a similar activity. In particular an increase of activity confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control.

In one embodiment, the homolog is a homolog with a sequence as indicated in Table I or II, column 7. In one embodiment, the homolog of one of the polypeptides indicated in Table II, column 3, is derived from an eukaryotic organism. In one embodiment, the homolog is derived from Fungi. In one embodiment, the homolog of a polypeptide indi-

cated in Table II, column 3, is derived from Ascomyceta. In one embodiment, the homolog of a polypeptide indicated in Table II, column 3 is derived from Saccharomycotina. In one embodiment, the homolog of a polypeptide indicated in Table II, column 3, is derived from Saccharomycetes, preferably from Saccharomycetales, from Saccharomycetaceae, more preferably from Saccharomycetes.

In one embodiment, the homolog is a homolog with a sequence as indicated in Table I or II, column 7. In one embodiment, the homolog of one of the polypeptides indicated in Table II, column 3, is derived from a bacteria. In one embodiment, the homolog of a polypeptide indicated in Table II, column 3, is derived from Proteobacteria.. In one embodiment, the homolog of a polypeptide indicated in Table II, column 3, is a homolog having the same or a similar activity being derived from Gammaproteobacteria, preferably from Enterobacteriales, from Enterobacteriaceae, more preferably from Escherichia.

In one embodiment the invention relates to homologs of the aforementioned sequences, which can be isolated advantageously from yeast, fungi, viruses, algae, bacteria, such as *Acetobacter* (subgen. *Acetobacter*) *aceti*; *Acidithiobacillus ferrooxidans*; *Acinetobacter* sp.; *Actinobacillus* sp; *Aeromonas salmonicida*; *Agrobacterium tumefaciens*; *Aquifex aeolicus*; *Arcanobacterium pyogenes*; *Aster yellows phytoplasma*; *Bacillus* sp.; *Bifidobacterium* sp.; *Borrelia burgdorferi*; *Brevibacterium linens*; *Brucella melitensis*; *Buchnera* sp.; *Butyrivibrio fibrisolvens*; *Campylobacter jejuni*; *Caulobacter crescentus*; *Chlamydia* sp.; *Chlamydophila* sp.; *Chlorobium limicola*; *Citrobacter rodentium*; *Clostridium* sp.; *Comamonas testosteroni*; *Corynebacterium* sp.; *Coxiella burnetii*; *Deinococcus radiodurans*; *Dichelobacter nodosus*; *Edwardsiella ictaluri*; *Enterobacter* sp.; *Erysipelothrix rhusiopathiae*; *Escherichia coli*; *Flavobacterium* sp.; *Francisella tularensis*; *Frankia* sp. Cpl1; *Fusobacterium nucleatum*; *Geobacillus stearothermophilus*; *Gluconobacter oxydans*; *Haemophilus* sp.; *Helicobacter pylori*; *Klebsiella pneumoniae*; *Lactobacillus* sp.; *Lactococcus lactis*; *Listeria* sp.; *Mannheimia haemolytica*; *Mesorhizobium loti*; *Methylophaga thalassica*; *Microcystis aeruginosa*; *Microscilla* sp. PRE1; *Moraxella* sp. TA144; *Mycobacterium* sp.; *Mycoplasma* sp.; *Neisseria* sp.; *Nitrosomonas* sp.; *Nostoc* sp. PCC 7120; *Novosphingobium aromaticivorans*; *Oenococcus oeni*; *Pantoea citrea*; *Pasteurella multocida*; *Pediococcus pentosaceus*; *Phormidium foveolarum*; *Phytoplasma* sp.; *Plectonema boryanum*; *Prevotella ruminicola*; *Propionibacterium* sp.; *Proteus vulgaris*; *Pseudomonas* sp.; *Ralstonia* sp.; *Rhizobium* sp.; *Rhodococcus equi*; *Rhodothermus marinus*; *Rickettsia* sp.; *Riemerella anatipestifer*; *Ruminococcus flavefaciens*; *Salmonella* sp.; *Selenomonas ruminantium*; *Serratia entomophila*; *Shigella* sp.; *Sinorhizobium meliloti*; *Staphylococcus* sp.; *Streptococcus* sp.; *Streptomyces* sp.; *Synechococcus* sp.; *Synechocystis* sp. PCC 6803; *Thermotoga maritima*; *Treponema* sp.; *Ureaplasma urealyticum*; *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Xylella fastidiosa*; *Yersinia* sp.; *Zymomonas mobilis*, preferably *Salmonella* sp. or *Escherichia coli* or plants, preferably from yeasts such as from the genera *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Torulopsis* or *Schizosaccharomyces* or

plants such as *Arabidopsis thaliana*, maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, borage, sunflower, linseed, primrose, rapeseed, canola and turnip rape, manihot, pepper, sunflower, tagetes, solanaceous plant such as potato, tobacco, eggplant and tomato, *Vicia* species, pea, alfalfa, bushy plants such as coffee, cacao, tea, *Salix* species, trees such as oil palm, coconut, perennial grass, such as ryegrass and fescue, and forage crops, such as alfalfa and clover and from spruce, pine or fir for example. More preferably homologs of aforementioned sequences can be isolated from *Saccharomyces cerevisiae*, *E. coli* or plants, preferably *Brassica napus*, *Glycine max*, *Zea mays*, cotton, or *Oryza sativa*.

- 10 **[0089.1.1.1]** The (stress related) proteins of the present invention are preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector, for example in to a binary vector, the expression vector is introduced into a host cell, for example the *Arabidopsis thaliana* wild type NASC N906 or any other plant cell as described in the examples see below, and the stress related protein is expressed in said host cell. Examples for binary vectors are pBIN19, pBI101, pBinAR, pGPTV, pCAMBIA, pBIB-HYG, pBecks, pGreen or pPZP (Hajukiewicz, P. et al., 1994, Plant Mol. Biol., 25: 989-994 and Hellens et al, Trends in Plant Science (2000) 5, 446-451.).

20 In one embodiment the (BSRP) protein of the present invention is preferably produced in an compartment of the cell, more preferably in the plastids. Ways of introducing nucleic acids into plastids and producing proteins in this compartment are known to the person skilled in the art have been also described in this application.

- 25 **[0090.1.1.1]** Advantageously, the nucleic acid sequences according to the invention or the gene construct together with at least one reporter gene are cloned into an expression cassette, which is introduced into the organism via a vector or directly into the genome. This reporter gene should allow easy detection via a growth, fluorescence, chemical, bioluminescence or resistance assay or via a photometric measurement. Examples of reporter genes which may be mentioned are antibiotic- or herbicide-resistance genes, hydrolase genes, fluorescence protein genes, bioluminescence genes, sugar or nucleotide metabolic genes or biosynthesis genes such as the *Ura3* gene, the *Ilv2* gene, the luciferase gene, the β -galactosidase gene, the *gfp* gene, the 2-desoxyglucose-6-phosphate phosphatase gene, the β -glucuronidase gene, β -lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene, a mutated acetohydroxyacid synthase (AHAS) gene, also known as acetolactate synthase (ALS) gene], a gene for a D-amino acid metabolizing enzyme or the BASTA (= glyphosate-resistance) gene. These genes permit easy measurement and quantification of the transcription activity and hence of the expression of the genes. In this way genome positions may be identified which exhibit differing productivity.

- 40 **[0091.1.1.1]** In a preferred embodiment a nucleic acid construct, for example an expression cassette, comprises upstream, i.e. at the 5' end of the encoding sequence,

a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and optionally other regulatory elements which are operably linked to the intervening encoding sequence with one of the nucleic acids of SEQ ID NO as depicted in table I, column 5 and 7. By an operable linkage is meant the sequential arrangement of promoter, encoding
 5 sequence, terminator and optionally other regulatory elements in such a way that each of the regulatory elements can fulfill its function in the expression of the encoding sequence in due manner. The sequences preferred for operable linkage are targeting sequences for ensuring subcellular localization in plastids. However, targeting sequences for ensuring subcellular localization in the mitochondrion, in the endoplasmic
 10 reticulum (= ER), in the nucleus, in oil corpuscles or other compartments may also be employed as well as translation promoters such as the 5' lead sequence in tobacco mosaic virus (Gallie et al., Nucl. Acids Res. 15 (1987), 8693 -8711).

[0092.1.1.1] A nucleic acid construct, for example an expression cassette may, for example, contain a constitutive promoter or a tissue-specific promoter (preferably the
 15 USP or napin promoter) the gene to be expressed and the ER retention signal. For the ER retention signal the KDEL amino acid sequence (lysine, aspartic acid, glutamic acid, leucine) or the KKX amino acid sequence (lysine-lysine-X-stop, wherein X means every other known amino acid) is preferably employed.

[0093.1.1.1] For expression in a host organism, for example a plant, the expression
 20 cassette is advantageously inserted into a vector such as by way of example a plasmid, a phage or other DNA which allows optimal expression of the genes in the host organism. Examples of suitable plasmids are: in E. coli pLG338, pACYC184, pBR series such as e.g. pBR322, pUC series such as pUC18 or pUC19, M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III¹¹³-B1,
 25 λ gt11 or pBdCl; in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361; in Bacillus pUB110, pC194 or pBD214; in Corynebacterium pSA77 or pAJ667; in fungi pALS1, pIL2 or pBB116; other advantageous fungal vectors are described by Romanos, M.A. et al., [(1992) „Foreign gene expression in yeast: a review“, *Yeast* 8: 423-488] and by van den Hondel, C.A.M.J.J. et al. [(1991) „Heterologous gene expression in filamentous
 30 fungi“ as well as in *More Gene Manipulations in Fungi* [J.W. Bennet & L.L. Lasure, eds., pp. 396-428: Academic Press: San Diego] and in „Gene transfer systems and vector development for filamentous fungi“ [van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) in: *Applied Molecular Genetics of Fungi*, Peberdy, J.F. et al., eds., pp. 1-28, Cambridge University Press: Cambridge]. Examples of advantageous yeast promoters
 35 are 2 μ M, pAG-1, YEpl6, YEpl13 or pEMBLYe23. Examples of algal or plant promoters are pLGV23, pGHIac⁺, pBIN19, pAK2004, pVKH or pDH51 (see Schmidt, R. and Willmitzer, L., 1988). The vectors identified above or derivatives of the vectors identified above are a small selection of the possible plasmids. Further plasmids are well known to those skilled in the art and may be found, for example, in the book *Cloning
 40 Vectors* (Eds. Pouwels P.H. et al. Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Suitable plant vectors are described inter alia in „Methods in

Plant Molecular Biology and Biotechnology" (CRC Press), Ch. 6/7, pp. 71-119. Advantageous vectors are known as shuttle vectors or binary vectors which replicate in *E. coli* and *Agrobacterium*.

- [0094.1.1.1]** By vectors is meant with the exception of plasmids all other vectors known to those skilled in the art such as by way of example phages, viruses such as SV40, CMV, baculovirus, adenovirus, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA. These vectors can be replicated autonomously in the host organism or be chromosomally replicated, chromosomal replication being preferred.
- [0095.1.1.1]** In a further embodiment of the vector the expression cassette according to the invention may also advantageously be introduced into the organisms in the form of a linear DNA and be integrated into the genome of the host organism by way of heterologous or homologous recombination. This linear DNA may be composed of a linearized plasmid or only of the expression cassette as vector or the nucleic acid sequences according to the invention.
- [0096.1.1.1]** In a further advantageous embodiment the nucleic acid sequence according to the invention can also be introduced into an organism on its own.
- [0097.1.1.1]** If in addition to the nucleic acid sequence according to the invention further genes are to be introduced into the organism, all together with a reporter gene in a single vector or each single gene with a reporter gene in a vector in each case can be introduced into the organism, whereby the different vectors can be introduced simultaneously or successively.
- [0098.1.1.1]** The vector advantageously contains at least one copy of the nucleic acid sequences according to the invention and/or the expression cassette (= gene construct) according to the invention.
- [0099.1.1.1]** The invention further provides an isolated recombinant expression vector comprising a nucleic acid encoding a polypeptide as depicted in table II, column 5 or 7, wherein expression of the vector in a host cell results in increased tolerance to environmental stress as compared to a wild type variety of the host cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell or an organelle upon introduction into the host cell, and thereby are replicated along with the host or organelle genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as

“expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression
5 vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses), which serve equivalent functions.

[0100.1.1.1] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory
10 sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. As used herein with respect to a recombinant expression vector, “operatively linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro*
15 transcription/translation system or in a host cell when the vector is introduced into the host cell). The term “regulatory sequence” is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) and Gruber
20 and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, eds. Glick and Thompson, Chapter 7, 89-108, CRC Press: Boca Raton, Florida, including the references therein. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will
25 be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides or peptides, encoded by nucleic acids as described herein (e.g., BSRP,
30 mutant forms of BSRP, fusion polypeptides, etc.).

[0101.1.1.1] The recombinant expression vectors of the invention can be designed for expression of the polypeptide of the invention in plant cells. For example, BSRP genes can be expressed in plant cells (See Schmidt, R. and Willmitzer, L., 1988, High efficiency *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana*
35 leaf and cotyledon explants, Plant Cell Rep. 583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, S.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. Kung und R. Wu, 128-43, Academic Press: 1993; Potrykus, 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42:205-225 and references cited therein).
40 Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press: San Diego, CA (1990). Alternatively, the

recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0102.1.1.1] Expression of polypeptides in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide but also to the C-terminus or fused within suitable regions in the polypeptides. Such fusion vectors typically serve three purposes: 1) to increase expression of a recombinant polypeptide; 2) to increase the solubility of a recombinant polypeptide; and 3) to aid in the purification of a recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin, and enterokinase.

[0103.1.1.1] By way of example the plant expression cassette can be installed in the pRT transformation vector ((a) Toepfer et al., 1993, *Methods Enzymol.*, 217: 66-78; (b) Toepfer et al. 1987, *Nucl. Acids. Res.* 15: 5890 ff.).

Alternatively, a recombinant vector (= expression vector) can also be transcribed and translated *in vitro*, e.g. by using the T7 promoter and the T7 RNA polymerase.

[0104.1.1.1] Expression vectors employed in prokaryotes frequently make use of inducible systems with and without fusion proteins or fusion oligopeptides, wherein these fusions can ensue in both N-terminal and C-terminal manner or in other useful domains of a protein. Such fusion vectors usually have the following purposes: i.) to increase the RNA expression rate; ii.) to increase the achievable protein synthesis rate; iii.) to increase the solubility of the protein; iv.) or to simplify purification by means of a binding sequence usable for affinity chromatography. Proteolytic cleavage points are also frequently introduced via fusion proteins, which allow cleavage of a portion of the fusion protein and purification. Such recognition sequences for proteases are recognized, e.g. factor Xa, thrombin and enterokinase.

[0105.1.1.1] Typical advantageous fusion and expression vectors are pGEX [Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67: 31-40], pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which contains glutathione S-transferase (GST), maltose binding protein or protein A.

[0106.1.1.1] In one embodiment, the coding sequence of the polypeptide of the invention is cloned into a pGEX expression vector to create a vector encoding a fusion polypeptide comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-X polypeptide. The fusion polypeptide can be purified by affinity chromatography using glutathione-agarose resin. Recombinant BSRP unfused to GST can be recovered by cleavage of the fusion polypeptide with thrombin.

Other examples of *E. coli* expression vectors are pTrc [Amann et al., (1988) *Gene* 69:301-315] and pET vectors [Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89; Stratagene, Amsterdam, The Netherlands].

- 5 **[0107.1.1.1]** Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 *gn10*-lac fusion promoter mediated by a co-expressed viral RNA polymerase (T7 *gn1*). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring
- 10 a T7 *gn1* gene under the transcriptional control of the lacUV 5 promoter.

- [0108.1.1.1]** In a preferred embodiment of the present invention, the BSRP are expressed in plants and plants cells such as unicellular plant cells (e.g. algae) (See Falcatore et al., 1999, *Marine Biotechnology* 1(3):239-251 and references therein) and plant cells from higher plants (e.g., the spermatophytes, such as crop plants). A nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 may be “introduced” into a plant cell by any means, including transfection, transformation or transduction, electroporation, particle bombardment, agroinfection, and the like. One transformation method known to those of skill in the art is the dipping of a flowering plant into an *Agrobacteria* solution, wherein the *Agrobacteria* contains the nucleic acid of the
- 15 invention, followed by breeding of the transformed gametes.
- 20

- [0109.1.1.1]** Other suitable methods for transforming or transfecting host cells including plant cells can be found in Sambrook, et al., *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and other laboratory manuals such as *Methods in Molecular Biology*, 1995, Vol. 44, *Agrobacterium* protocols, ed: Gartland and Davey, Humana Press, Totowa, New Jersey. As biotic and abiotic stress tolerance is a general trait wished to be inherited into a wide variety of plants like maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed and canola, manihot, pepper, sunflower and tagetes, solanaceous plants like potato, tobacco, eggplant, and tomato,
- 25 Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), *Salix* species, trees (oil palm, coconut), perennial grasses, and forage crops, these crop plants are also preferred target plants for a genetic engineering as one further embodiment of the present invention. Forage crops include, but are not limited to, Wheatgrass, Canarygrass, Bromegrass, Wildrye Grass, Bluegrass, Orchardgrass, Alfalfa, Salfoin, Birdsfoot Trefoil,
- 30 Alsike Clover, Red Clover, and Sweet Clover.
- 35

- [0110.1.1.1]** In one embodiment of the present invention, transfection of a nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 into a plant is achieved by *Agrobacterium* mediated gene transfer. *Agrobacterium* mediated plant transformation can be performed using for example the GV3101(pMP90) (Koncz and Schell, 1986, *Mol. Gen. Genet.* 204:383-396) or LBA4404 (Clontech) *Agrobacterium tumefaciens* strain. Transformation can be performed by standard transformation and
- 40

regeneration techniques (Deblaere et al., 1994, Nucl. Acids Res. 13:4777-4788; Gelvin, Stanton B. and Schilperoort, Robert A, Plant Molecular Biology Manual, 2nd Ed. - Dordrecht : Kluwer Academic Publ., 1995. - in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R.; Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993 360 S., ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989, Plant cell Report 8:238-242; De Block et al., 1989, Plant Physiol. 91:694-701). Use of antibiotics for *Agrobacterium* and plant selection depends on the binary vector and the *Agrobacterium* strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. *Agrobacterium* mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994, Plant Cell Report 13:282-285. Additionally, transformation of soybean can be performed using for example a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent No. 0397 687, U.S. Patent No. 5,376,543, or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbide fiber technique. (See, for example, Freeling and Walbot "The maize handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387, and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

[0111.1.1.1] According to the present invention, the introduced nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes or organelle genome. Alternatively, the introduced BSRP may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active.

[0112.1.1.1] In one embodiment, a homologous recombinant microorganism can be created wherein the BSRP is integrated into a chromosome, a vector is prepared which contains at least a portion of a nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 into which a deletion, addition, or substitution has been introduced to thereby alter, e.g., functionally disrupt, the BSRP gene. Preferably, the BSRP gene is a yeast or a *E.coli* gene, but it can be a homolog from a related plant or even from a mammalian or insect source. The vector can be designed such that, upon homologous recombination, the endogenous nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 is mutated or otherwise altered but still encodes a functional polypeptide (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous BSRP). In a preferred embodiment the biological activity of the protein of the invention is increased upon homologous recombination. To create a point mutation via homologous recombination, DNA-RNA hybrids can be used in a technique known as chimeraplasty (Cole-Strauss et al., 1999, Nucleic Acids

Research 27(5):1323-1330 and Kmiec, 1999 Gene therapy American Scientist. 87(3):240-247). Homologous recombination procedures in *Physcomitrella patens* are also well known in the art and are contemplated for use herein.

[0113.1.1.1] Whereas in the homologous recombination vector, the altered portion of the nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 is flanked at its 5' and 3' ends by an additional nucleic acid molecule of the BSRP gene to allow for homologous recombination to occur between the exogenous BSRP gene carried by the vector and an endogenous BSRP gene, in a microorganism or plant. The additional flanking BSRP nucleic acid molecule is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several hundreds of base pairs up to kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See, e.g., Thomas, K.R., and Capecchi, M.R., 1987, Cell 51:503 for a description of homologous recombination vectors or Strepp et al., 1998, PNAS, 95 (8):4368-4373 for cDNA based recombination in *Physcomitrella patens*). The vector is introduced into a microorganism or plant cell (e.g., via polyethylene glycol mediated DNA), and cells in which the introduced BSRP gene has homologously recombined with the endogenous BSRP gene are selected using art-known techniques.

[0114.1.1.1] Whether present in an extra-chromosomal non-replicating vector or a vector that is integrated into a chromosome, the nucleic acid molecule coding for BSRP as depicted in table II, column 5 or 7 preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from *Agrobacterium tumefaciens* t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited on transcriptional levels, a plant expression cassette preferably contains other operatively linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the polypeptide per RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). Examples of plant expression vectors include those detailed in: Becker, D. et al., 1992, New plant binary vectors with selectable markers located proximal to the left border, Plant Mol. Biol. 20: 1195-1197; and Bevan, M.W., 1984, Binary *Agrobacterium* vectors for plant transformation, Nucl. Acid. Res. 12:8711-8721; and Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds.: Kung and R. Wu, Academic Press, 1993, S. 15-38.

[0115.1.1.1] "Transformation" is defined herein as a process for introducing heterologous DNA into a plant cell, plant tissue, or plant. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may

rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time. Transformed plant cells, plant tissue, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof.

- 10 The terms "transformed," "transgenic," and "recombinant" refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A "non-transformed," "non-transgenic," or "non-recombinant" host refers to a wild-type organism, e.g., a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

A "transgenic plant", as used herein, refers to a plant which contains a foreign nucleotide sequence inserted into either its nuclear genome or organellar genome. It encompasses further the offspring generations i.e. the T1-, T2- and consecutively generations or BC1-, BC2- and consecutively generation as well as crossbreeds thereof with non-transgenic or other transgenic plants.

- [0116.1.1.1]** The host organism (= transgenic organism) advantageously contains at least one copy of the nucleic acid according to the invention and/or of the nucleic acid construct according to the invention.

In principle all plants can be used as host organism. Preferred transgenic plants are, for example, selected from the families Aceraceae, Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Cactaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Nymphaeaceae, Papaveraceae, Rosaceae, Salicaceae, Solanaceae, Arecaceae, Bromeliaceae, Cyperaceae, Iridaceae, Liliaceae, Orchidaceae, Gentianaceae, Labiaceae, Magnoliaceae, Ranunculaceae, Carifolaceae, Rubiaceae, Scrophulariaceae, Caryophyllaceae, Ericaceae, Polygonaceae, Violaceae, Juncaceae or Poaceae and preferably from a plant selected from the group of the families Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Papaveraceae, Rosaceae, Solanaceae, Liliaceae or Poaceae. Preferred are crop plants such as plants advantageously selected from the group of the genus peanut, oilseed rape, canola, sunflower, safflower, olive, sesame, hazelnut, almond, avocado, bay, pumpkin/squash, linseed, soya, pistachio, borage, maize, wheat, rye, oats, sorghum and millet, triticale, rice, barley, cassava, potato, sugarbeet, egg plant, alfalfa, and perennial grasses and forage plants, oil palm, vegetables (brassicas, root vegetables, tuber vegetables, pod vegeta-

bles, fruiting vegetables, onion vegetables, leafy vegetables and stem vegetables), buckwheat, Jerusalem artichoke, broad bean, vetches, lentil, dwarf bean, lupin, clover and Lucerne for mentioning only some of them.

In one embodiment of the invention transgenic plants are selected from the group
5 comprising corn, soy, oil seed rape (including canola and winter oil seed reape), cotton, wheat and rice.

In one preferred embodiment, the host plant is selected from the families Aceraceae, Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Cactaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Nymphaeaceae, Papaveraceae, Rosaceae, Salicaceae, Solanaceae, Arecaceae, Bromeliaceae, Cyperaceae, Iridaceae, Liliaceae,
10 Orchidaceae, Gentianaceae, Labiaceae, Magnoliaceae, Ranunculaceae, Carifolaceae, Rubiaceae, Scrophulariaceae, Caryophyllaceae, Ericaceae, Polygonaceae, Violaceae, Juncaceae or Poaceae and preferably from a plant selected from the group of the families Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Papaveraceae,
15 Rosaceae, Solanaceae, Liliaceae or Poaceae. Preferred are crop plants and in particular plants mentioned herein above as host plants such as the families and genera mentioned above for example preferred the species *Anacardium occidentale*, *Calendula officinalis*, *Carthamus tinctorius*, *Cichorium intybus*, *Cynara scolymus*, *Helianthus annuus*, *Tagetes lucida*, *Tagetes erecta*, *Tagetes tenuifolia*; *Daucus carota*; *Corylus avellana*, *Corylus colurna*, *Borago officinalis*; *Brassica napus*, *Brassica rapa* ssp., *Sinapis arvensis* *Brassica juncea*, *Brassica juncea* var. *juncea*, *Brassica juncea* var. *crispifolia*, *Brassica juncea* var. *foliosa*, *Brassica nigra*, *Brassica sinapioides*, *Melanosinapis communis*, *Brassica oleracea*, *Arabidopsis thaliana*, *Anana comosus*, *Ananas ananas*, *Bromelia comosa*, *Carica papaya*, *Cannabis sativa*, *Ipomoea batatas*, *Ipomoea pandurata*, *Convolvulus batatas*, *Convolvulus tiliaceus*, *Ipomoea fastigiata*, *Ipomoea tiliacea*,
20 *Ipomoea triloba*, *Convolvulus panduratus*, *Beta vulgaris*, *Beta vulgaris* var. *altissima*, *Beta vulgaris* var. *vulgaris*, *Beta maritima*, *Beta vulgaris* var. *perennis*, *Beta vulgaris* var. *conditiva*, *Beta vulgaris* var. *esculenta*, *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita pepo*, *Cucurbita moschata*, *Olea europaea*, *Manihot utilissima*, *Janipha manihot*,
25 *Jatropha manihot*, *Manihot aipil*, *Manihot dulcis*, *Manihot manihot*, *Manihot melanobasis*, *Manihot esculenta*, *Ricinus communis*, *Pisum sativum*, *Pisum arvense*, *Pisum humile*, *Medicago sativa*, *Medicago falcata*, *Medicago varia*, *Glycine max* *Dolichos soja*, *Glycine gracilis*, *Glycine hispida*, *Phaseolus max*, *Soja hispida*, *Soja max*, *Cocos nucifera*, *Pelargonium grossularioides*, *Oleum cocoas*, *Laurus nobilis*, *Persea americana*, *Arachis hypogaea*, *Linum usitatissimum*, *Linum humile*, *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum flavum*, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*, *Linum perenne*, *Linum perenne* var. *lewisii*, *Linum pratense*, *Linum trigynum*, *Punica granatum*, *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum*,
30 *Gossypium thurberi*, *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa* spp., *Elaeis guineensis*, *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium*, *Sesamum*

- indicum, Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata*, , Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon *Hordeum aegiceras*, *Hordeum hexastichon.*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, Hordeum secalinum, Avena sativa, Avena fatua, *Avena byzantina*, *Avena fatua* var. *sativa*, *Avena hybrida*, Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*, *Sorghum miliaceum* millet, Panicum militaceum, Zea mays, Triticum aestivum, Triticum durum, Triticum turgidum, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare*, Coffea spp., Coffea arabica, Coffea canephora, Coffea liberica, Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens, Capsicum annuum, Nicotiana tabacum, Solanum tuberosum, Solanum melongena, Lycopersicon esculentum, *Lycopersicon lycopersicum.*, *Lycopersicon pyriforme*, Solanum integrifolium, *Solanum lycopersicum* Theobroma cacao or Camellia sinensis.
- 20 Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium e.g. the species Pistacia vera [pistachios, Pistazie], Mangifer indica [Mango] or Anacardium occidentale [Cashew]; Asteraceae such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana e.g. the species Calendula officinalis [Marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [blue daisy], Cynara scolymus [Artichoke], Helianthus annuus [sunflower], Lactuca sativa, *Lactuca crispa*, *Lactuca esculenta*, *Lactuca scariola* L. ssp. *sativa*, *Lactuca scariola* L. var. *integrata*, *Lactuca scariola* L. var. *integrifolia*, *Lactuca sativa* subsp. *romana*, *Locusta communis*, *Valeriana locusta* [lettuce], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [Marigold]; Apiaceae such as the genera
- 30 Daucus e.g. the species Daucus carota [carrot]; Betulaceae such as the genera Corylus e.g. the species Corylus avellana or Corylus colurna [hazelnut]; Boraginaceae such as the genera Borago e.g. the species Borago officinalis [borage]; Brassicaceae such as the genera Brassica, Melanosinapis, Sinapis, Arabidopsis e.g. the species Brassica napus, Brassica rapa ssp. [canola, oilseed rape, turnip rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, *Brassica sinapioides*, *Melanosinapis communis* [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana; Bromeliaceae such as the genera Anana, Bromelia e.g. the species Anana comosus, *Ananas ananas* or *Bromelia comosa* [pineapple]; Caricaceae such as the genera Carica e.g. the species Carica
- 40 papaya [papaya]; Cannabaceae such as the genera Cannabis e.g. the species Cannabis sativa [hemp], Convolvulaceae such as the genera Ipomea, Convolvulus e.g. the species Ipomoea batatas, Ipomoea pandurata, *Convolvulus batatas*, *Convolvulus tilia-*

ceus, *Ipomoea fastigiata*, *Ipomoea tiliacea*, *Ipomoea triloba* or *Convolvulus panduratus*
 [sweet potato, Man of the Earth, wild potato], Chenopodiaceae such as the genera
 Beta, i.e. the species *Beta vulgaris*, *Beta vulgaris* var. *altissima*, *Beta vulgaris* var. *Vul-*
 5 *vularis*, *Beta maritima*, *Beta vulgaris* var. *perennis*, *Beta vulgaris* var. *conditiva* or *Beta*
vulgaris var. *esculenta* [sugar beet]; Cucurbitaceae such as the genera *Cucurbita* e.g.
 the species *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita pepo* or *Cucurbita moschata*
 [pumpkin, squash]; Elaeagnaceae such as the genera *Elaeagnus* e.g. the species *Olea*
europaea [olive]; Ericaceae such as the genera *Kalmia* e.g. the species *Kalmia latifolia*,
Kalmia angustifolia, *Kalmia microphylla*, *Kalmia polifolia*, *Kalmia occidentalis*, *Cistus*
 10 *chamaerhodendros* or *Kalmia lucida* [American laurel, broad-leafed laurel, calico bush,
 spoon wood, sheep laurel, alpine laurel, bog laurel, western bog-laurel, swamp-laurel];
 Euphorbiaceae such as the genera *Manihot*, *Janipha*, *Jatropha*, *Ricinus* e.g. the spe-
 cies *Manihot utilissima*, *Janipha manihot*, *Jatropha manihot*, *Manihot aipil*, *Manihot*
dulcis, *Manihot manihot*, *Manihot melanobasis*, *Manihot esculenta* [manihot, arrowroot,
 15 tapioca, cassava] or *Ricinus communis* [castor bean, Castor Oil Bush, Castor Oil Plant,
 Palma Christi, Wonder Tree]; Fabaceae such as the genera *Pisum*, *Albizia*, *Cathormi-*
on, *Feuillea*, *Inga*, *Pithecolobium*, *Acacia*, *Mimosa*, *Medicago*, *Glycine*, *Dolichos*,
Phaseolus, *Soja* e.g. the species *Pisum sativum*, *Pisum arvense*, *Pisum humile* [pea],
Albizia berteriana, *Albizia julibrissin*, *Albizia lebbek*, *Acacia berteriana*, *Acacia littora-*
 20 *lis*, *Albizia berteriana*, *Albizia berteriana*, *Cathormion berteriana*, *Feuillea berteriana*,
Inga fragrans, *Pithecellobium berterianum*, *Pithecellobium fragrans*, *Pithecolobium ber-*
terianum, *Pseudalbizia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*, *Feu-*
illea julibrissin, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericanrda julibrissin*, *Acacia*
lebbeck, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea lebbeck*, *Mimosa lebbeck*, *Mi-*
 25 *mosa speciosa* [bastard logwood, silk tree, East Indian Walnut], *Medicago sativa*, *Me-*
dicago falcata, *Medicago varia* [alfalfa] *Glycine max* *Dolichos soja*, *Glycine gracilis*,
Glycine hispida, *Phaseolus max*, *Soja hispida* or *Soja max* [soybean]; Geraniaceae
 such as the genera *Pelargonium*, *Cocos*, *Oleum* e.g. the species *Cocos nucifera*, *Pe-*
largonium grossularioides or *Oleum cocois* [coconut]; Gramineae such as the genera
 30 *Saccharum* e.g. the species *Saccharum officinarum*; Juglandaceae such as the genera
Juglans, *Wallia* e.g. the species *Juglans regia*, *Juglans ailanthifolia*, *Juglans sieboldia-*
na, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*, *Juglans hindsii*,
Juglans intermedia, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans*
nigra or *Wallia nigra* [walnut, black walnut, common walnut, persian walnut, white wal-
 35 nut, butternut, black walnut]; Lauraceae such as the genera *Persea*, *Laurus* e.g. the
 species *laurel Laurus nobilis* [bay, laurel, bay laurel, sweet bay], *Persea americana*
Persea americana, *Persea gratissima* or *Persea persea* [avocado]; Leguminosae such
 as the genera *Arachis* e.g. the species *Arachis hypogaea* [peanut]; Linaceae such as
 the genera *Linum*, *Adenolinum* e.g. the species *Linum usitatissimum*, *Linum humile*,
 40 *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum fla-*
vum, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*,
Linum perenne, *Linum perenne* var. *lewisii*, *Linum pratense* or *Linum trigynum* [flax,

- linseed]; Lythraeae such as the genera Punica e.g. the species Punica granatum [pomegranate]; Malvaceae such as the genera Gossypium e.g. the species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton]; Musaceae such as the genera Musa e.g. the species
- 5 Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana]; Onagraceae such as the genera Camissonia, Oenothera e.g. the species Oenothera biennis or Camissonia brevipes [primrose, evening primrose]; Palmae such as the genera Elaeis e.g. the species Elaeis guineensis [oil palm]; Papaveraceae such as the genera Papaver e.g. the species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* [poppy,
- 10 oriental poppy, corn poppy, field poppy, shirley poppies, field poppy, long-headed poppy, long-pod poppy]; Pedaliaceae such as the genera Sesamum e.g. the species Sesamum indicum [sesame]; Piperaceae such as the genera Piper, Artanthe, Peperomia, Steffensia e.g. the species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata*. [Cayenne pepper, wild pepper]; Poaceae such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea, Triticum e.g. the species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, Hordeum secalinum [barley, pearl barley, foxtail barley, wall barley, meadow barley], Secale cereale [rye], Avena sativa, Avena fatua, *Avena byzantina*, *Avena fatua var. sativa*, *Avena hybrida* [oat], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*, *Sorghum miliaceum* millet, Panicum miliaceum [*Sorghum*, millet], Oryza sativa, Oryza latifolia [rice], Zea mays [corn,
- 25 maize] Triticum aestivum, Triticum durum, Triticum turgidum, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* [wheat, bread wheat, common wheat], Proteaceae such as the genera Macadamia e.g. the species Macadamia integrifolia [macadamia]; Rubiaceae such as the genera Coffea e.g. the species Coffea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee]; Scrophulariaceae
- 35 such as the genera Verbascum e.g. the species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein, white moth mullein, nettle-leaved mullein, dense-flowered mullein, silver mullein, long-leaved mullein, white mullein, dark mullein, greek mullein, orange mullein, purple mullein,
- 40 hoary mullein, great mullein]; Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon e.g. the species Capsicum annuum, Capsicum annuum

var. glabriusculum, *Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*, *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [egg-plant] (*Lycopersicon esculentum*, *Lycopersicon lycopersicum*., *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* [tomato]; Sterculiaceae such as the genera *Theobroma* e.g. the species *Theobroma cacao* [cacao]; Theaceae such as the genera *Camellia* e.g. the species *Camellia sinensis*) [tea].

[0117.1.1.1] The introduction of the nucleic acids according to the invention, the expression cassette or the vector into organisms, plants for example, can in principle be done by all of the methods known to those skilled in the art. The introduction of the nucleic acid sequences gives rise to recombinant or transgenic organisms.

[0118.1.1.1] Unless otherwise specified, the terms "polynucleotides", "nucleic acid" and "nucleic acid molecule" as used herein are interchangeably. Unless otherwise specified, the terms "peptide", "polypeptide" and "protein" are interchangeably in the present context. The term "sequence" may relate to polynucleotides, nucleic acids, nucleic acid molecules, peptides, polypeptides and proteins, depending on the context in which the term "sequence" is used. The terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid molecule(s)" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. The terms refer only to the primary structure of the molecule.

[0119.1.1.1] Thus, the terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", or "nucleic acid molecule(s)" as used herein include double- and single-stranded DNA and RNA. They also include known types of modifications, for example, methylation, "caps", substitutions of one or more of the naturally occurring nucleotides with an analog. Preferably, the DNA or RNA sequence of the invention comprises a coding sequence encoding the herein defined polypeptide.

[0119.2.1.1] The genes of the invention, coding for an activity selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein are also called "BSRP gene".

[0120.1.1.1] A "coding sequence" is a nucleotide sequence, which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropri-

ate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

[0121.1.1.1] The transfer of foreign genes into the genome of a plant is called transformation. In doing this the methods described for the transformation and regeneration of plants from plant tissues or plant cells are utilized for transient or stable transformation. Suitable methods are protoplast transformation by poly(ethylene glycol)-induced DNA uptake, the „biolistic“ method using the gene cannon – referred to as the particle bombardment method, electroporation, the incubation of dry embryos in DNA solution, microinjection and gene transfer mediated by *Agrobacterium*. Said methods are described by way of example in B. Jenes et al., *Techniques for Gene Transfer*, in: *Transgenic Plants*, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 42 (1991) 205-225). The nucleic acids or the construct to be expressed is preferably cloned into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., *Nucl. Acids Res.* 12 (1984) 8711). *Agrobacteria* transformed by such a vector can then be used in known manner for the transformation of plants, in particular of crop plants such as by way of example tobacco plants, for example by bathing bruised leaves or chopped leaves in an *agrobacterial* solution and then culturing them in suitable media. The transformation of plants by means of *Agrobacterium tumefaciens* is described, for example, by Höfgen and Willmitzer in *Nucl. Acid Res.* (1988) 16, 9877 or is known inter alia from F.F. White, *Vectors for Gene Transfer in Higher Plants*; in *Transgenic Plants*, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

[0122.1.1.1] *Agrobacteria* transformed by an expression vector according to the invention may likewise be used in known manner for the transformation of plants such as test plants like *Arabidopsis* or crop plants such as cereal crops, corn, oats, rye, barley, wheat, soybean, rice, cotton, sugar beet, canola, sunflower, flax, hemp, potatoes, tobacco, tomatoes, carrots, paprika, oilseed rape, tapioca, cassava, arrowroot, tagetes, alfalfa, lettuce and the various tree, nut and vine species, in particular of oil-containing crop plants such as soybean, peanut, castor oil plant, sunflower, corn, cotton, flax, oilseed rape, coconut, oil palm, safflower (*Carthamus tinctorius*) or cocoa bean, e.g. by bathing bruised leaves or chopped leaves in an *agrobacterial* solution and then culturing them in suitable media.

The genetically modified plant cells may be regenerated by all of the methods known to those skilled in the art. Appropriate methods can be found in the publications referred to above by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

[0123.1.1.1] Accordingly, a further aspect of the invention relates to transgenic organisms transformed by at least one nucleic acid sequence, expression cassette or

vector according to the invention as well as cells, cell cultures, tissue, parts – such as, for example, leaves, roots, etc. in the case of plant organisms – or reproductive material derived from such organisms. The terms „host organism“, „host cell“, „recombinant (host) organism“ and „transgenic (host) cell“ are used here interchangeably. Of course these terms relate not only to the particular host organism or the particular target cell but also to the descendants or potential descendants of these organisms or cells. Since, due to mutation or environmental effects certain modifications may arise in successive generations, these descendants need not necessarily be identical with the parental cell but nevertheless are still encompassed by the term as used here.

For the purposes of the invention „transgenic“ or „recombinant“ means with regard for example to a nucleic acid sequence, an expression cassette (= gene construct, nucleic acid construct) or a vector containing the nucleic acid sequence according to the invention or an organism transformed by the nucleic acid sequences, expression cassette or vector according to the invention all those constructions produced by genetic engineering methods in which either

- a) the nucleic acid sequence depicted in table I, column 5 or 7 or its derivatives or parts thereof or
- b) a genetic control sequence functionally linked to the nucleic acid sequence described under (a), for example a 3'- and/or 5'- genetic control sequence such as a promoter or terminator, or
- c) (a) and (b)

are not found in their natural, genetic environment or have been modified by genetic engineering methods, wherein the modification may by way of example be a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment means the natural genomic or chromosomal locus in the organism of origin or inside the host organism or presence in a genomic library. In the case of a genomic library the natural genetic environment of the nucleic acid sequence is preferably retained at least in part. The environment borders the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, particularly preferably at least 1,000 bp, most particularly preferably at least 5,000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequence according to the invention with the corresponding delta-8-desaturase, delta-9-elongase and/or delta-5-desaturase gene – turns into a transgenic expression cassette when the latter is modified by unnatural, synthetic („artificial“) methods such as by way of example a mutagenation. Appropriate methods are described by way of example in US 5,565,350 or WO 00/15815.

[0124.1.1.1] Suitable organisms or host organisms for the nucleic acid, expression cassette or vector according to the invention are advantageously in principle all organisms, which are suitable for the expression of recombinant genes as described above.

Further examples which may be mentioned are plants such as Arabidopsis, Asteraceae such as Calendula or crop plants such as soybean, peanut, castor oil plant, sunflower, flax, corn, cotton, flax, oilseed rape, coconut, oil palm, safflower (*Carthamus tinctorius*) or cocoa bean.

- 5 In one embodiment of the invention host plants for the nucleic acid, expression cassette or vector according to the invention are selected from the group comprising corn, soy, oil seed rape (including canola and winter oil seed reap), cotton, wheat and rice.

[0125.1.1.1] A further object of the invention relates to the use of a nucleic acid construct, e.g. an expression cassette, containing DNA sequences encoding polypeptides
10 shown in table II or DNA sequences hybridizing therewith for the transformation of plant cells, tissues or parts of plants.

In doing so, depending on the choice of promoter, the sequences of shown in table I can be expressed specifically in the leaves, in the seeds, the nodules, in roots, in the stem or other parts of the plant. Those transgenic plants overproducing sequences as
15 depicted in table I, the reproductive material thereof, together with the plant cells, tissues or parts thereof are a further object of the present invention.

The expression cassette or the nucleic acid sequences or construct according to the invention containing sequences according to table I can, moreover, also be employed for the transformation of the organisms identified by way of example above such as
20 bacteria, yeasts, filamentous fungi and plants.

[0126.1.1.1] Within the framework of the present invention, increased resistance to biotic stress, preferably pathogenic fungi means, for example, the artificially acquired trait of increased resistance to biotic stress, preferably pathogenic fungi due to functional expression or over expression of polypeptide sequences of table II encoded by
25 the corresponding nucleic acid molecules as depicted in table I, column 5 or 7 and/or homologs in the organisms according to the invention, advantageously in the transgenic plants according to the invention, by comparison with the nongenetically modified initial plants at least for the duration of at least one plant generation.

[0127.1.1.1] A constitutive expression of the polypeptide sequences of the of table II encoded by the corresponding nucleic acid molecule as depicted in table I, column 5 or
30 7 and/or homologs is, moreover, advantageous. On the other hand, however, an inducible expression may also appear desirable. Expression of the polypeptide sequences of the invention can be either accurate "direct" in the cytoplasm, eg from a nucleus transformed or encoded gene, or in the organelles preferably the plastids of the
35 host cells, preferably the plant cells.

The efficiency of the expression of the sequences of the of table II encoded by the corresponding nucleic acid molecule as depicted in table I, column 5 or 7 and/or homologs can be determined, for example, in vitro by shoot meristem propagation. In addition, an expression of the sequences of of table II encoded by the corresponding
40 nucleic acid molecule as depicted in table I, column 5 or 7 and/or homologs modified

in nature and level and its effect on the stress resistance can be tested on test plants in greenhouse trials.

[0128.1.1.1] An additional object of the invention comprises transgenic organisms such as transgenic plants transformed by an expression cassette containing sequences of as depicted in table I, column 5 or 7 according to the invention or DNA sequences hybridizing therewith, as well as transgenic cells, tissue, parts and reproduction material of such plants. Particular preference is given in this case to transgenic crop plants such as by way of example barley, wheat, rye, oats, corn, soybean, rice, cotton, sugar beet, oilseed rape and canola, sunflower, flax, hemp, thistle, potatoes, tobacco, tomatoes, tapioca, cassava, arrowroot, alfalfa, lettuce and the various tree, nut and vine species.

In one embodiment of the invention transgenic plants transformed by an expression cassette containing sequences of as depicted in table I, column 5 or 7 according to the invention or DNA sequences hybridizing therewith are selected from the group comprising corn, soy, oil seed rape (including canola and winter oil seed rape), cotton, wheat and rice.

[0129.1.1.1] For the purposes of the invention plants are mono- and dicotyledonous plants, mosses or algae.

A further refinement according to the invention are transgenic plants as described above which contain a nucleic acid sequence or construct according to the invention or a expression cassette according to the invention.

[0129.2.1.1] However, transgenic also means that the nucleic acids according to the invention are located at their natural position in the genome of an organism, but that the sequence has been modified in comparison with the natural sequence and/or that the regulatory sequences of the natural sequences have been modified. Preferably, transgenic/recombinant is to be understood as meaning the transcription of the nucleic acids of the invention and shown in table I, occurs at a non-natural position in the genome, that is to say the expression of the nucleic acids is homologous or, preferably, heterologous. This expression can be transiently or of a sequence integrated stably into the genome.

The term "transgenic plants" used in accordance with the invention also refers to the progeny of a transgenic plant, for example the T_1 , T_2 , T_3 and subsequent plant generations or the BC_1 , BC_2 , BC_3 and subsequent plant generations. Thus, the transgenic plants according to the invention can be raised and selfed or crossed with other individuals in order to obtain further transgenic plants according to the invention. Transgenic plants may also be obtained by propagating transgenic plant cells vegetatively. The present invention also relates to transgenic plant material, which can be derived from a transgenic plant population according to the invention. Such material includes plant cells and certain tissues, organs and parts of plants in all their manifestations, such as seeds, leaves, anthers, fibers, tubers, roots, root hairs, stems, embryo, calli,

cotyledons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures, which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant.

Any transformed plant obtained according to the invention can be used in a conventional breeding scheme or in in vitro plant propagation to produce more transformed plants with the same characteristics and/or can be used to introduce the same characteristic in other varieties of the same or related species. Such plants are also part of the invention. Seeds obtained from the transformed plants genetically also contain the same characteristic and are part of the invention. As mentioned before, the present invention is in principle applicable to any plant and crop that can be transformed with any of the transformation method known to those skilled in the art.

[0130.1.1.1] Advantageous inducible plant promoters are by way of example the PRP1 promoter [Ward et al., Plant.Mol. Biol.22(1993), 361-366], a promoter inducible by benzenesulfonamide (EP 388 186), a promoter inducible by tetracycline [Gatz et al., (1992) Plant J. 2,397-404], a promoter inducible by salicylic acid (WO 95/19443), a promoter inducible by abscisic acid (EP 335 528) and a promoter inducible by ethanol or cyclohexanone (WO93/21334). Other examples of plant promoters which can advantageously be used are the promoter of cytosolic FBPase from potato, the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8 (1989) 2445-245), the promoter of phosphoribosyl pyrophosphate amidotransferase from Glycine max (see also gene bank accession number U87999) or a nodiene-specific promoter as described in EP 249 676. Particular advantageous are those promoters which ensure expression expression upon the early onset of environmental stress like for example drought or cold or biotic stress caused by plant diseases.

In one embodiment seed-specific promoters may be used for monocotyledonous or dicotyledonous plants.

[0131.1.1.1] In principle all natural promoters with their regulation sequences can be used like those named above for the expression cassette according to the invention and the method according to the invention. Over and above this, synthetic promoters may also advantageously be used.

In the preparation of an expression cassette various DNA fragments can be manipulated in order to obtain a nucleotide sequence, which usefully reads in the correct direction and is equipped with a correct reading frame. To connect the DNA fragments (= nucleic acids according to the invention) to one another adaptors or linkers may be attached to the fragments.

The promoter and the terminator regions can usefully be provided in the transcription direction with a linker or polylinker containing one or more restriction points for the insertion of this sequence. Generally, the linker has 1 to 10, mostly 1 to 8, preferably 2 to 6, restriction points. In general the size of the linker inside the regulatory region is less than 100 bp, frequently less than 60 bp, but at least 5 bp. The promoter may be both

native or homologous as well as foreign or heterologous to the host organism, for example to the host plant. In the 5'-3' transcription direction the expression cassette contains the promoter, a DNA sequence which shown in table I and a region for transcription termination. Different termination regions can be exchanged for one another in any
5 desired fashion.

[0132.1.1.1] As also used herein, the terms "nucleic acid" and "nucleic acid molecule" are intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. This term also encompasses untranslated sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 1000 nucleotides of sequence upstream from the 5' end of the coding region and at least about 200 nucleotides of sequence downstream from the 3' end of the coding region of the gene. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

15 An "isolated" nucleic acid molecule is one that is substantially separated from other nucleic acid molecules, which are present in the natural source of the nucleic acid. That means other nucleic acid molecules are present in an amount less than 5% based on weight of the amount of the desired nucleic acid, preferably less than 2% by weight, more preferably less than 1% by weight, most preferably less than 0.5% by weight.

20 Preferably, an "isolated" nucleic acid is free of some of the sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated stress related protein encoding nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of

25 nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be free from some of the other cellular material with which it is naturally associated, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

30

[0133.1.1.1] A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule encoding an BSRP or a portion thereof which confers tolerance and/or resistance to biotic stress and preferably a further yield related trait in plants, can be isolated using standard molecular biological techniques and the sequence information
35 provided herein. For example, an *Arabidopsis thaliana* stress related protein encoding cDNA can be isolated from a *A. thaliana* c-DNA library or a *Synechocystis sp.*, *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* stress related protein encoding cDNA can be isolated from a *Synechocystis sp.*, *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* c-DNA library respectively using all or portion of one of the sequences
40 shown in table I. Moreover, a nucleic acid molecule encompassing all or a portion of one of the sequences of table I can be isolated by the polymerase chain reaction using

oligonucleotide primers designed based upon this sequence. For example, mRNA can be isolated from plant cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al., 1979 Biochemistry 18:5294-5299) and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from
5 Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon one of the nucleotide sequences shown in table I. A nucleic acid molecule of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleo-
10 tide primers according to standard PCR amplification techniques. The nucleic acid molecule so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to a BSRP encoding nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

15 In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises one of the nucleotide sequences shown in table I encoding the BSRP (i.e., the "coding region"), as well as 5' untranslated sequences and 3' untranslated sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the coding region of one of the sequences of the nucleic acid of table I, for example, a
20 fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a BSRP.

[0134.1.1.1] Portions of proteins encoded by the BSRP encoding nucleic acid molecules of the invention are preferably biologically active portions described herein. As used herein, the term "biologically active portion of" a BSRP is intended to include a
25 portion, e.g., a domain/motif, of stress related protein that participates in a stress tolerance and/or resistance response in a plant. To determine whether a BSRP, or a biologically active portion thereof, results in increased stress tolerance in a plant, a stress analysis of a plant comprising the BSRP may be performed. Such analysis methods are well known to those skilled in the art, as detailed in the Examples. More specifically,
30 nucleic acid fragments encoding biologically active portions of a BSRP can be prepared by isolating a portion of one of the sequences of the nucleic acid of table I expressing the encoded portion of the BSRP or peptide (e.g., by recombinant expression in vitro) and assessing the activity of the encoded portion of the BSRP or peptide.

Biologically active portions of a BSRP are encompassed by the present invention and
35 include peptides comprising amino acid sequences derived from the amino acid sequence of a BSRP encoding gene, or the amino acid sequence of a protein homologous to a BSRP, which include fewer amino acids than a full length BSRP or the full length protein which is homologous to a BSRP, and exhibits at least some enzymatic or biological activity of a BSRP. Typically, biologically active portions (e.g., peptides which
40 are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of a BSRP. Moreover,

other biologically active portions in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically active portions of a BSRP include one or more selected domains/motifs or portions thereof having biological activity.

- 5 The term "biological active portion" or "biological activity" means a polypeptide as depicted in table II, column 3 or a portion of said polypeptide which still has at least 10 % or 20 %, preferably 20 %, 30 %, 40 % or 50 %, especially preferably 60 %, 70 % or 80 % of the enzymatic or biological activity of the natural or starting enzyme or protein.

- 10 **[0135.1.1.1]** In the process according to the invention nucleic acid sequences can be used, which, if appropriate, contain synthetic, non-natural or modified nucleotide bases, which can be incorporated into DNA or RNA. Said synthetic, non-natural or modified bases can for example increase the stability of the nucleic acid molecule outside or inside a cell. The nucleic acid molecules of the invention can contain the same modifications as aforementioned.

- 15 **[0136.1.1.1]** As used in the present context the term "nucleic acid molecule" may also encompass the untranslated sequence located at the 3' and at the 5' end of the coding gene region, for example at least 500, preferably 200, especially preferably 100, nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, especially preferably 20, nucleotides of the sequence downstream
20 of the 3' end of the coding gene region. It is often advantageous only to choose the coding region for cloning and expression purposes.

[0137.1.1.1] Preferably, the nucleic acid molecule used in the process according to the invention or the nucleic acid molecule of the invention is an isolated nucleic acid molecule.

- 25 **[0138.1.1.1]** An "isolated" polynucleotide or nucleic acid molecule is separated from other polynucleotides or nucleic acid molecules, which are present in the natural source of the nucleic acid molecule. An isolated nucleic acid molecule may be a chromosomal fragment of several kb, or preferably, a molecule only comprising the coding region of the gene. Accordingly, an isolated nucleic acid molecule of the invention may
30 comprise chromosomal regions, which are adjacent 5' and 3' or further adjacent chromosomal regions, but preferably comprises no such sequences which naturally flank the nucleic acid molecule sequence in the genomic or chromosomal context in the organism from which the nucleic acid molecule originates (for example sequences which are adjacent to the regions encoding the 5'- and 3'-UTRs of the nucleic acid molecule).
35 In various embodiments, the isolated nucleic acid molecule used in the process according to the invention may, for example comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid molecule originates.

[0139.1.1.1] The nucleic acid molecules used in the process, for example the polynucleotide of the invention or of a part thereof can be isolated using molecular-biological standard techniques and the sequence information provided herein. Also, for example a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level can be identified with the aid of comparison algorithms. The former can be used as hybridization probes under standard hybridization techniques (for example those described in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences useful in this process.

[0140.1.1.1] A nucleic acid molecule encompassing a complete sequence of the nucleic acid molecules used in the process, for example the polynucleotide of the invention, or a part thereof may additionally be isolated by polymerase chain reaction, oligonucleotide primers based on this sequence or on parts thereof being used. For example, a nucleic acid molecule comprising the complete sequence or part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been generated on the basis of this very sequence. For example, mRNA can be isolated from cells (for example by means of the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be generated by means of reverse transcriptase (for example Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase, obtainable from Seikagaku America, Inc., St.Petersburg, FL).

[0141.1.1.1] Synthetic oligonucleotide primers for the amplification, e.g. as shown in table III, column 7, by means of polymerase chain reaction can be generated on the basis of a sequence shown herein, for example the sequence shown in table I, columns 5 and 7 or the sequences derived from table II, columns 5 and 7.

[0142.1.1.1] Moreover, it is possible to identify conserved protein by carrying out protein sequence alignments with the polypeptide encoded by the nucleic acid molecules of the present invention, in particular with the sequences encoded by the nucleic acid molecule shown in, column 5 or 7 of Table I, from which conserved regions, and in turn, degenerate primers can be derived.

Conserved regions are those, which show a very little variation in the amino acid in one particular position of several homologs from different origin. The consensus sequence and polypeptide motifs shown in column 7 of Table IV are derived from said alignments, are represent such conserved protein regions. Moreover, it is possible to identify conserved regions from various organisms by carrying out protein sequence alignments with the polypeptide encoded by the nucleic acid of the present invention, in particular with the sequences encoded by the polypeptide molecule shown in column 5 or 7 of Table II, from which conserved regions, and in turn, degenerate primers can be derived.

In one advantageous embodiment, in the method of the present invention the activity of a polypeptide is increased comprising or consisting of a consensus sequence or a polypeptide motif shown in table IV column 7 and in one another embodiment, the present invention relates to a polypeptide comprising or consisting of a consensus sequence or a polypeptide motif shown in table IV, column 7 whereby 20 or less, preferably 15 or 10, preferably 9, 8, 7, or 6, more preferred 5 or 4, even more preferred 3, even more preferred 2, even more preferred 1, most preferred 0 of the amino acids positions indicated can be replaced by any amino acid. In one embodiment not more than 15%, preferably 10%, even more preferred 5%, 4%, 3%, or 2%, most preferred 1% or 0% of the amino acid position indicated by a letter are/is replaced another amino acid. In one embodiment 20 or less, preferably 15 or 10, preferably 9, 8, 7, or 6, more preferred 5 or 4, even more preferred 3, even more preferred 2, even more preferred 1, most preferred 0 amino acids are inserted into a consensus sequence or protein motif.

The consensus sequence was derived from a multiple alignment of the sequences as listed in table II. The letters represent the one letter amino acid code and indicate that the amino acids are conserved in at least 80% of the aligned proteins, whereas the letter X stands for amino acids, which are not conserved in at least 80% of the aligned sequences. The consensus sequence starts with the first conserved amino acid in the alignment, and ends with the last conserved amino acid in the alignment of the investigated sequences. The number of given X indicates the distances between conserved amino acid residues, e.g. Y-x(21,23)-F means that conserved tyrosine and phenylalanine residues in the alignment are separated from each other by minimum 21 and maximum 23 amino acid residues in the alignment of all investigated sequences.

Conserved domains were identified from all sequences and are described using a subset of the standard Prosite notation, e.g the pattern Y-x(21,23)-[FW] means that a conserved tyrosine is separated by minimum 21 and maximum 23 amino acid residues from either a phenylalanine or tryptophane. Patterns had to match at least 80% of the investigated proteins.

Conserved patterns were identified with the software tool MEME version 3.5.1 or manually. MEME was developed by Timothy L. Bailey and Charles Elkan, Dept. of Computer Science and Engineering, University of California, San Diego, USA and is described by Timothy L. Bailey and Charles Elkan [Fitting a mixture model by expectation maximization to discover motifs in biopolymers, Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology, pp. 28-36, AAAI Press, Menlo Park, California, 1994]. The source code for the stand-alone program is public available from the San Diego Supercomputer center (<http://meme.sdsc.edu>).

For identifying common motifs in all sequences with the software tool MEME, the following settings were used: -maxsize 500000, -nmotifs 15, -evt 0.001, -maxw 60, -distance 1e-3, -minsites number of sequences used for the analysis. Input sequences for MEME were non-aligned sequences in Fasta format. Other parameters were used in the default settings in this software version.

Prosite patterns for conserved domains were generated with the software tool Pratt version 2.1 or manually. Pratt was developed by Inge Jonassen, Dept. of Informatics, University of Bergen, Norway and is described by Jonassen et al. [I. Jonassen, J.F. Collins and D.G. Higgins, Finding flexible patterns in unaligned protein sequences, Protein Science 4 (1995), pp. 1587-1595; I. Jonassen, Efficient discovery of conserved patterns using a pattern graph, Submitted to CABIOS Febr. 1997]. The source code (ANSI C) for the stand-alone program is public available, e.g. at established Bioinformatic centers like EBI (European Bioinformatics Institute).

For generating patterns with the software tool Pratt, following settings were used: PL (max Pattern Length): 100, PN (max Nr of Pattern Symbols): 100, PX (max Nr of consecutive x's): 30, FN (max Nr of flexible spacers): 5, FL (max Flexibility): 30, FP (max Flex.Product): 10, ON (max number patterns): 50. Input sequences for Pratt were distinct regions of the protein sequences exhibiting high similarity as identified from software tool MEME. The minimum number of sequences, which have to match the generated patterns (CM, min Nr of Seqs to Match) was set to at least 80% of the provided sequences. Parameters not mentioned here were used in their default settings.

The Prosite patterns of the conserved domains can be used to search for protein sequences matching this pattern. Various established Bioinformatic centers provide public internet portals for using those patterns in database searches (e.g. PIR [Protein Information Resource, located at Georgetown University Medical Center] or ExPASy [Expert Protein Analysis System]). Alternatively, stand-alone software is available, like the program Fuzzpro, which is part of the EMBOSS software package. For example, the program Fuzzpro not only allows to search for an exact pattern-protein match but also allows to set various ambiguities in the performed search.

The alignment was performed with the software ClustalW (version 1.83) and is described by Thompson et al. [Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680]. The source code for the stand-alone program is public available from the European Molecular Biology Laboratory; Heidelberg, Germany. The analysis was performed using the default parameters of ClustalW v1.83 (gap open penalty: 10.0; gap extension penalty: 0.2; protein matrix: Gonnet; pprotein/DNA endgap: -1; protein/DNA gapdist: 4).

[0143.1.1.1] Degenerated primers can then be utilized by PCR for the amplification of fragments of novel proteins having above-mentioned activity, e.g. conferring the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof after increasing the expression or activity or having the activity of a protein as shown in table II, column 3 or further functional homologs of the polypeptide of the invention from other organisms.

- [0144.1.1.1]** These fragments can then be utilized as hybridization probe for isolating the complete gene sequence. As an alternative, the missing 5' and 3' sequences can be isolated by means of RACE-PCR. A nucleic acid molecule according to the invention can be amplified using cDNA or, as an alternative, genomic DNA as template and suitable oligonucleotide primers, following standard PCR amplification techniques. The nucleic acid molecule amplified thus can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides, which correspond to one of the nucleic acid molecules used in the process can be generated by standard synthesis methods, for example using an automatic DNA synthesizer.
- [0145.1.1.1]** Nucleic acid molecules which are advantageously for the process according to the invention can be isolated based on their homology to the nucleic acid molecules disclosed herein using the sequences or part thereof as hybridization probe and following standard hybridization techniques under stringent hybridization conditions. In this context, it is possible to use, for example, isolated nucleic acid molecules of at least 15, 20, 25, 30, 35, 40, 50, 60 or more nucleotides, preferably of at least 15, 20 or 25 nucleotides in length which hybridize under stringent conditions with the above-described nucleic acid molecules, in particular with those which encompass a nucleotide sequence of the nucleic acid molecule used in the process of the invention or encoding a protein used in the invention or of the nucleic acid molecule of the invention. Nucleic acid molecules with 30, 50, 100, 250 or more nucleotides may also be used.
- [0146.1.1.1]** The term "homology" means that the respective nucleic acid molecules or encoded proteins are functionally and/or structurally equivalent. The nucleic acid molecules that are homologous to the nucleic acid molecules described above and that are derivatives of said nucleic acid molecules are, for example, variations of said nucleic acid molecules which represent modifications having the same biological function, in particular encoding proteins with the same or substantially the same biological function. They may be naturally occurring variations, such as sequences from other plant varieties or species, or mutations. These mutations may occur naturally or may be obtained by mutagenesis techniques. The allelic variations may be naturally occurring allelic variants as well as synthetically produced or genetically engineered variants. Structurally equivalents can, for example, be identified by testing the binding of said polypeptide to antibodies or computer based predictions. Structurally equivalent have the similar immunological characteristic, e.g. comprise similar epitopes.
- [0147.1.1.1]** By "hybridizing" it is meant that such nucleic acid molecules hybridize under conventional hybridization conditions, preferably under stringent conditions such as described by, e.g., Sambrook (Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)) or in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6.
- [0148.1.1.1]** According to the invention, DNA as well as RNA molecules of the nucleic acid of the invention can be used as probes. Further, as template for the identifi-

cation of functional homologues Northern blot assays as well as Southern blot assays can be performed. The Northern blot assay advantageously provides further informations about the expressed gene product: e.g. expression pattern, occurrence of processing steps, like splicing and capping, etc. The Southern blot assay provides additional information about the chromosomal localization and organization of the gene encoding the nucleic acid molecule of the invention.

[0149.1.1.1] A preferred, nonlimiting example of stringent hybridization conditions are hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C, for example at 50°C, 55°C or 60°C. The skilled worker knows that these hybridization conditions differ as a function of the type of the nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. The temperature under "standard hybridization conditions" differs for example as a function of the type of the nucleic acid between 42°C and 58°C, preferably between 45°C and 50°C in an aqueous buffer with a concentration of 0.1 x 0.5 x, 1 x, 2x, 3x, 4x or 5 x SSC (pH 7.2). If organic solvent(s) is/are present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 40°C, 42°C or 45°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 x SSC and 20°C, 25°C, 30°C, 35°C, 40°C or 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are preferably for example 0.1 x SSC and 30°C, 35°C, 40°C, 45°C, 50°C or 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows to determine the hybridization conditions required with the aid of textbooks, for example the ones mentioned above, or from the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

[0150.1.1.1] A further example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for one hour. Alternatively, an exemplary stringent hybridization condition is in 50 % formamide, 4XSSC at 42°C. Further, the conditions during the wash step can be selected from the range of conditions delimited by low-stringency conditions (approximately 2X SSC at 50°C) and high-stringency conditions (approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3M sodium citrate, 3M NaCl, pH 7.0). In addition, the temperature during the wash step can be raised from low-stringency conditions at room temperature, approximately 22°C, to higher-stringency conditions at approximately 65°C. Both of the parameters salt concentration and temperature can be varied simultaneously, or else one of the two parameters can be kept constant while only the other is varied. De-

naturants, for example formamide or SDS, may also be employed during the hybridization. In the presence of 50% formamide, hybridization is preferably effected at 42°C. Relevant factors like i) length of treatment, ii) salt conditions, iii) detergent conditions, iv) competitor DNAs, v) temperature and vi) probe selection can be combined case by case so that not all possibilities can be mentioned herein.

Thus, in a preferred embodiment, Northern blots are prehybridized with Rothi-Hybri-Quick buffer (Roth, Karlsruhe) at 68°C for 2h. Hybridization with radioactive labelled probe is done overnight at 68°C. Subsequent washing steps are performed at 68°C with 1xSSC.

For Southern blot assays the membrane is prehybridized with Rothi-Hybri-Quick buffer (Roth, Karlsruhe) at 68°C for 2h. The hybridization with radioactive labelled probe is conducted over night at 68°C. Subsequently the hybridization buffer is discarded and the filter shortly washed using 2xSSC; 0,1% SDS. After discarding the washing buffer new 2xSSC; 0,1% SDS buffer is added and incubated at 68°C for 15 minutes. This washing step is performed twice followed by an additional washing step using 1xSSC; 0,1% SDS at 68°C for 10 min.

[0151.1.1.1] Some examples of conditions for DNA hybridization (Southern blot assays) and wash step are shown hereinbelow:

- (1) Hybridization conditions can be selected, for example, from the following conditions:
 - a) 4X SSC at 65°C,
 - b) 6X SSC at 45°C,
 - c) 6X SSC, 100 mg/ml denatured fragmented fish sperm DNA at 68°C,
 - d) 6X SSC, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA at 68°C,
 - e) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA, 50% formamide at 42°C,
 - f) 50% formamide, 4X SSC at 42°C,
 - g) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrate at 42°C,
 - h) 2X or 4X SSC at 50°C (low-stringency condition), or
 - i) 30 to 40% formamide, 2X or 4X SSC at 42°C (low-stringency condition).
- (2) Wash steps can be selected, for example, from the following conditions:
 - a) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.
 - b) 0.1X SSC at 65°C.
 - c) 0.1X SSC, 0.5 % SDS at 68°C.
 - d) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C.
 - e) 0.2X SSC, 0.1% SDS at 42°C.
 - f) 2X SSC at 65°C (low-stringency condition).

[0152.1.1.1] Polypeptides having above-mentioned activity, i.e. conferring the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof, derived from other organisms, can be encoded by other DNA sequences which hybridize to the sequences shown in table I, columns 5 and 7 under relaxed hybridization conditions and which code on expression for peptides conferring the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof.

[0153.1.1.1] Further, some applications have to be performed at low stringency hybridisation conditions, without any consequences for the specificity of the hybridisation. For example, a Southern blot analysis of total DNA could be probed with a nucleic acid molecule of the present invention and washed at low stringency (55°C in 2xSSPE, 0,1% SDS). The hybridisation analysis could reveal a simple pattern of only genes encoding polypeptides of the present invention or used in the process of the invention, e.g. having herein-mentioned activity of increasing the tolerance and/or resistance to biotic stress and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof. A further example of such low-stringent hybridization conditions is 4XSSC at 50°C or hybridization with 30 to 40% formamide at 42°C. Such molecules comprise those which are fragments, analogues or derivatives of the polypeptide of the invention or used in the process of the invention and differ, for example, by way of amino acid and/or nucleotide deletion(s), insertion(s), substitution (s), addition(s) and/or recombination (s) or any other modification(s) known in the art either alone or in combination from the above-described amino acid sequences or their underlying nucleotide sequence(s). However, it is preferred to use high stringency hybridisation conditions.

[0154.1.1.1] Hybridization should advantageously be carried out with fragments of at least 5, 10, 15, 20, 25, 30, 35 or 40 bp, advantageously at least 50, 60, 70 or 80 bp, preferably at least 90, 100 or 110 bp. Most preferably are fragments of at least 15, 20, 25 or 30 bp. Preferably are also hybridizations with at least 100 bp or 200, very especially preferably at least 400 bp in length. In an especially preferred embodiment, the hybridization should be carried out with the entire nucleic acid sequence with conditions described above.

[0155.1.1.1] The terms "fragment", "fragment of a sequence" or "part of a sequence" mean a truncated sequence of the original sequence referred to. The truncated sequence (nucleic acid or protein sequence) can vary widely in length; the minimum size being a sequence of sufficient size to provide a sequence with at least a comparable function and/or activity of the original sequence referred to or hybridizing with the nucleic acid molecule of the invention or used in the process of the invention under stringent conditions, while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired activity and/or function(s) of the original sequence.

[0156.1.1.1] Typically, the truncated amino acid sequence will range from about 5 to about 310 amino acids in length. More typically, however, the sequence will be a maximum of about 250 amino acids in length, preferably a maximum of about 200 or 100 amino acids. It is usually desirable to select sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

[0157.1.1.1] The term "epitope" relates to specific immunoreactive sites within an antigen, also known as antigenic determinates. These epitopes can be a linear array of monomers in a polymeric composition – such as amino acids in a protein – or consist of or comprise a more complex secondary or tertiary structure. Those of skill will recognize that immunogens (i.e., substances capable of eliciting an immune response) are antigens; however, some antigen, such as haptens, are not immunogens but may be made immunogenic by coupling to a carrier molecule. The term "antigen" includes references to a substance to which an antibody can be generated and/or to which the antibody is specifically immunoreactive.

[0158.1.1.1] In one embodiment the present invention relates to a epitope of the polypeptide of the present invention or used in the process of the present invention and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof.

[0159.1.1.1] The term "one or several amino acids" relates to at least one amino acid but not more than that number of amino acids, which would result in a homology of below 50% identity. Preferably, the identity is more than 70% or 80%, more preferred are 85%, 90%, 91%, 92%, 93%, 94% or 95%, even more preferred are 96%, 97%, 98%, or 99% identity.

[0160.1.1.1] Further, the nucleic acid molecule of the invention comprises a nucleic acid molecule, which is a complement of one of the nucleotide sequences of above mentioned nucleic acid molecules or a portion thereof. A nucleic acid molecule which is complementary to one of the nucleotide sequences shown in table I, columns 5 and 7 is one which is sufficiently complementary to one of the nucleotide sequences shown in table I, columns 5 and 7 such that it can hybridize to one of the nucleotide sequences shown in table I, columns 5 and 7, thereby forming a stable duplex. Preferably, the hybridisation is performed under stringent hybridization conditions. However, a complement of one of the herein disclosed sequences is preferably a sequence complement thereto according to the base pairing of nucleic acid molecules well known to the skilled person. For example, the bases A and G undergo base pairing with the bases T and U or C, resp. and visa versa. Modifications of the bases can influence the base-pairing partner.

[0161.1.1.1] The nucleic acid molecule of the invention comprises a nucleotide sequence which is at least about 30%, 35%, 40% or 45%, preferably at least about 50%, 55%, 60% or 65%, more preferably at least about 70%, 80%, or 90%, and even more

preferably at least about 95%, 97%, 98%, 99% or more homologous to a nucleotide sequence shown in table I, columns 5 and 7, or a portion thereof and preferably has above mentioned activity, in particular having a tolerance and/or resistance to biotic stress and preferably a further yield related trait increasing activity after increasing the activity or an activity of a gene product as shown in table II, column 3 by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids.

[0162.1.1.1] The nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes, preferably hybridizes under stringent conditions as defined herein, to one of the nucleotide sequences shown in table I, columns 5 and 7, or a portion thereof and encodes a protein having above-mentioned activity, e.g. conferring an increased resistance to biotic stress, preferably pathogenic fungi and preferably a further yield related trait as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids, and optionally, the activity selected from the group consisting of: b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, chorismate mutase-T and prephenate dehydrogenase, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit, transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein.

[0163.1.1.1] Moreover, the nucleic acid molecule of the invention can comprise only a portion of the coding region of one of the sequences shown in table I, columns 5 and 7, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of the polypeptide of the present invention or of a polypeptide used in the process of the present invention, i.e. having above-mentioned activity, e.g. conferring an increase of the tolerance and/or resistance to biotic stress and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof if its activity is increased by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids. The nucleotide sequences determined from the cloning of the present protein-according-to-the-invention-encoding gene allows for the generation of probes and primers designed for use in identifying and/or cloning its homologues in other cell types and organisms. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 15 preferably about 20 or 25, more preferably about 40, 50 or 75 consecutive nucleo-

tides of a sense strand of one of the sequences set forth, e.g., in table I, columns 5 and 7, an anti-sense sequence of one of the sequences, e.g., set forth in table I, columns 5 and 7, or naturally occurring mutants thereof. Primers based on a nucleotide of invention can be used in PCR reactions to clone homologues of the polypeptide of the invention or of the polypeptide used in the process of the invention, e.g. as the primers described in the examples of the present invention, e.g. as shown in the examples. A PCR with the primers shown in table III, column 7 will result in a fragment of the gene product as shown in table II, column 3.

[0164.1.1.1] Primer sets are interchangeable. The person skilled in the art knows to combine said primers to result in the desired product, e.g. in a full length clone or a partial sequence. Probes based on the sequences of the nucleic acid molecule of the invention or used in the process of the present invention can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. The probe can further comprise a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a genomic marker test kit for identifying cells which express an polypeptide of the invention or used in the process of the present invention, such as by measuring a level of an encoding nucleic acid molecule in a sample of cells, e.g., detecting mRNA levels or determining, whether a genomic gene comprising the sequence of the polynucleotide of the invention or used in the process of the present invention has been mutated or deleted.

[0165.1.1.1] The nucleic acid molecule of the invention encodes a polypeptide or portion thereof which includes an amino acid sequence which is sufficiently homologous to the amino acid sequence shown in table II, columns 5 and 7 such that the protein or portion thereof maintains the ability to participate in the increase of tolerance and/or resistance to biotic stress and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof, in particular increasing the activity as mentioned above or as described in the examples in plants is comprised.

[0166.1.1.1] As used herein, the language "sufficiently homologous" refers to proteins or portions thereof which have amino acid sequences which include a minimum number of identical or equivalent amino acid residues (e.g., an amino acid residue which has a similar side chain as an amino acid residue in one of the sequences of the polypeptide of the present invention) to an amino acid sequence shown in table II, columns 5 and 7 such that the protein or portion thereof is able to participate in the increase of the increase of tolerance and/or resistance to stress, the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type plant cell, plant or part thereof. For examples having the activity of a protein as shown in table II, column 3 and as described herein.

[0167.1.1.1] In one embodiment, the nucleic acid molecule of the present invention comprises a nucleic acid that encodes a portion of the protein of the present invention.

The protein is at least about 30%, 35%, 40%, 45% or 50%, preferably at least about 55%, 60%, 65% or 70%, and more preferably at least about 75%, 80%, 85%, 90%, 91%, 92%, 93% or 94% and most preferably at least about 95%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of table II, columns 5 and 7 and

5 having above-mentioned activity, e.g. conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids.

10 **[0168.1.1.1]** Portions of proteins encoded by the nucleic acid molecule of the invention are preferably biologically active, preferably having above-mentioned annotated activity, e.g. conferring an increase in tolerance and/or resistance to stress, the increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type plant cell, plant or part thereof after increase
15 of activity.

[0169.1.1.1] As mentioned herein, the term "biologically active portion" is intended to include a portion, e.g., a domain/motif, that confers increase in tolerance and/or resistance to biotic stress and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof or has an immunological activity such that it binds to an antibody binding specifically to the polypeptide of the present invention or a polypeptide used in the process of the present invention for increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof.
20

[0170.1.1.1] The invention further relates to nucleic acid molecules that differ from one of the nucleotide sequences shown in table I A, columns 5 and 7 (and portions thereof) due to degeneracy of the genetic code and thus encode a polypeptide of the present invention, in particular a polypeptide having above mentioned activity, e.g. as that polypeptides depicted by the sequence shown in table II, columns 5 and 7 or the functional homologues. Advantageously, the nucleic acid molecule of the invention
25 comprises, or in an other embodiment has, a nucleotide sequence encoding a protein comprising, or in an other embodiment having, an amino acid sequence shown in table II, columns 5 and 7 or the functional homologues. In a still further embodiment, the nucleic acid molecule of the invention encodes a full length protein which is substantially homologous to an amino acid sequence shown in table II, columns 5 and 7 or the
30 functional homologues. However, in a preferred embodiment, the nucleic acid molecule of the present invention does not consist of the sequence shown in table I, preferably table IA, columns 5 and 7.

[0171.1.1.1] In addition, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences may exist
40 within a population. Such genetic polymorphism in the gene encoding the polypeptide

of the invention or comprising the nucleic acid molecule of the invention may exist among individuals within a population due to natural variation.

[0172.1.1.1] As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding the polypeptide of the invention or comprising the nucleic acid molecule of the invention or encoding the polypeptide used in the process of the present invention, preferably from a crop plant or from a microorganism useful for the method of the invention. Such natural variations can typically result in 1-5% variance in the nucleotide sequence of the gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in genes encoding a polypeptide of the invention or comprising the nucleic acid molecule of the invention that are the result of natural variation and that do not alter the functional activity as described are intended to be within the scope of the invention.

[0173.1.1.1] Nucleic acid molecules corresponding to natural variants homologues of a nucleic acid molecule of the invention, which can also be a cDNA, can be isolated based on their homology to the nucleic acid molecules disclosed herein using the nucleic acid molecule of the invention, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

[0174.1.1.1] Accordingly, in another embodiment, a nucleic acid molecule of the invention is at least 15, 20, 25 or 30 nucleotides in length. Preferably, it hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of the nucleic acid molecule of the present invention or used in the process of the present invention, e.g. comprising the sequence shown in table I, columns 5 and 7. The nucleic acid molecule is preferably at least 20, 30, 50, 100, 250 or more nucleotides in length.

[0175.1.1.1] The term "hybridizes under stringent conditions" is defined above. In one embodiment, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 30 %, 40 %, 50 % or 65% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 75% or 80%, and even more preferably at least about 85%, 90% or 95% or more identical to each other typically remain hybridized to each other.

[0176.1.1.1] Preferably, nucleic acid molecule of the invention that hybridizes under stringent conditions to a sequence shown in table I, columns 5 and 7 corresponds to a naturally-occurring nucleic acid molecule of the invention. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). Preferably, the nucleic acid molecule encodes a natural protein having above-mentioned activity, e.g. conferring the tolerance and/or resistance to biotic stress and preferably a further yield related trait increase after increasing the expression or activity thereof or the activity of a protein of the invention or used in the process of the invention by for example ex-

pression the nucleic acid sequence of the gene product in the cytosol and/or in an organelle such as a plastid or mitochondria, preferably in plastids.

[0177.1.1.1] In addition to naturally-occurring variants of the sequences of the polypeptide or nucleic acid molecule of the invention as well as of the polypeptide or nucleic acid molecule used in the process of the invention that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into a nucleotide sequence of the nucleic acid molecule encoding the polypeptide of the invention or used in the process of the present invention, thereby leading to changes in the amino acid sequence of the encoded said polypeptide, without altering the functional ability of the polypeptide, preferably not decreasing said activity.

[0178.1.1.1] For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of the nucleic acid molecule of the invention or used in the process of the invention, e.g. shown in table I, columns 5 and 7.

[0179.1.1.1] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one without altering the activity of said polypeptide, whereas an "essential" amino acid residue is required for an activity as mentioned above, e.g. leading to an increase in the tolerance and/or resistance to biotic stress and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof in an organism after an increase of activity of the polypeptide. Other amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having said activity) may not be essential for activity and thus are likely to be amenable to alteration without altering said activity.

[0180.1.1.1] Further, a person skilled in the art knows that the codon usage between organisms can differ. Therefore, he may adapt the codon usage in the nucleic acid molecule of the present invention to the usage of the organism or the cell compartment for example of the plastid or mitochondria in which the polynucleotide or polypeptide is expressed.

[0181.1.1.1] Accordingly, the invention relates to nucleic acid molecules encoding a polypeptide having above-mentioned activity, in an organism or parts thereof by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids that contain changes in amino acid residues that are not essential for said activity. Such polypeptides differ in amino acid sequence from a sequence contained in the sequences shown in table II, columns 5 and 7 yet retain said activity described herein. The nucleic acid molecule can comprise a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 50% identical to an amino acid sequence shown in table II, columns 5 and 7 and is capable of participation in the increased biotic stress resistance and preferably a further yield related trait as compared to a corresponding non-

transformed wild type plant cell, plant or part thereof after increasing its activity, e.g. its expression by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% identical to the sequence shown in table II, columns 5 and 7, more preferably at least about 70% identical to one of the sequences shown in table II, columns 5 and 7, even more preferably at least about 80%, 90%, 95% homologous to the sequence shown in table II, columns 5 and 7, and most preferably at least about 96%, 97%, 98%, or 99% identical to the sequence shown in table II, columns 5 and 7.

- 10 **[0182.1.1.1]** To determine the percentage homology (= identity, herein used interchangeably) of two amino acid sequences or of two nucleic acid molecules, the sequences are written one underneath the other for an optimal comparison (for example gaps may be inserted into the sequence of a protein or of a nucleic acid in order to generate an optimal alignment with the other protein or the other nucleic acid).
- 15 **[0183.1.1.1]** The amino acid residues or nucleic acid molecules at the corresponding amino acid positions or nucleotide positions are then compared. If a position in one sequence is occupied by the same amino acid residue or the same nucleic acid molecule as the corresponding position in the other sequence, the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity". The percentage homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e. % homology = number of identical positions/total number of positions x 100). The terms "homology" and "identity" are thus to be considered as synonyms.
- 20 **[0184.1.1.1]** For the determination of the percentage homology (=identity) of two or more amino acids or of two or more nucleotide sequences several computer software programs have been developed. The homology of two or more sequences can be calculated with for example the software fasta, which presently has been used in the version fasta 3 (W. R. Pearson and D. J. Lipman (1988), Improved Tools for Biological Sequence Comparison.PNAS 85:2444- 2448; W. R. Pearson (1990) Rapid and Sensitive Sequence Comparison with FASTP and FASTA, Methods in Enzymology 183:63 - 98; W. R. Pearson and D. J. Lipman (1988) Improved Tools for Biological Sequence Comparison.PNAS 85:2444- 2448; W. R. Pearson (1990); Rapid and Sensitive Sequence Comparison with FASTP and FASTA Methods in Enzymology 183:63 - 98).
- 30 Another useful program for the calculation of homologies of different sequences is the standard blast program, which is included in the Biomax pedant software (Biomax, Munich, Federal Republic of Germany). This leads unfortunately sometimes to suboptimal results since blast does not always include complete sequences of the subject and the query. Nevertheless as this program is very efficient it can be used for the comparison of a huge number of sequences. The following settings are typically used for such a comparisons of sequences:
- 40

- p Program Name [String]; -d Database [String]; default = nr; -i Query File [File In]; default = stdin; -e Expectation value (E) [Real]; default = 10.0; -m alignment view options: 0 = pairwise; 1 = query-anchored showing identities; 2 = query-anchored no identities; 3 = flat query-anchored, show identities; 4 = flat query-anchored, no identities; 5 = query-anchored no identities and blunt ends; 6 = flat query-anchored, no identities and blunt ends; 7 = XML Blast output; 8 = tabular; 9 tabular with comment lines [Integer]; default = 0; -o BLAST report Output File [File Out] Optional; default = stdout; -F Filter query sequence (DUST with blastn, SEG with others) [String]; default = T; -G Cost to open a gap (zero invokes default behavior) [Integer]; default = 0; -E Cost to extend a gap (zero invokes default behavior) [Integer]; default = 0; -X X dropoff value for gapped alignment (in bits) (zero invokes default behavior); blastn 30, megablast 20, tblastx 0, all others 15 [Integer]; default = 0; -l Show GI's in defines [T/F]; default = F; -q Penalty for a nucleotide mismatch (blastn only) [Integer]; default = -3; -r Reward for a nucleotide match (blastn only) [Integer]; default = 1; -v Number of database sequences to show one-line descriptions for (V) [Integer]; default = 500; -b Number of database sequence to show alignments for (B) [Integer]; default = 250; -f Threshold for extending hits, default if zero; blastp 11, blastn 0, blastx 12, tblastn 13; tblastx 13, megablast 0 [Integer]; default = 0; -g Perform gapped alignment (not available with tblastx) [T/F]; default = T; -Q Query Genetic code to use [Integer]; default = 1; -D DB Genetic code (for tblast[nx] only) [Integer]; default = 1; -a Number of processors to use [Integer]; default = 1; -O SeqAlign file [File Out] Optional; -J Believe the query define [T/F]; default = F; -M Matrix [String]; default = BLOSUM62; -W Word size, default if zero (blastn 11, megablast 28, all others 3) [Integer]; default = 0; -z Effective length of the database (use zero for the real size) [Real]; default = 0; -K Number of best hits from a region to keep (off by default, if used a value of 100 is recommended) [Integer]; default = 0; -P 0 for multiple hit, 1 for single hit [Integer]; default = 0; -Y Effective length of the search space (use zero for the real size) [Real]; default = 0; -S Query strands to search against database (for blast[nx], and tblastx); 3 is both, 1 is top, 2 is bottom [Integer]; default = 3; -T Produce HTML output [T/F]; default = F; -l Restrict search of database to list of GI's [String] Optional; -U Use lower case filtering of FASTA sequence [T/F] Optional; default = F; -y X dropoff value for ungapped extensions in bits (0.0 invokes default behavior); blastn 20, megablast 10, all others 7 [Real]; default = 0.0; -Z X dropoff value for final gapped alignment in bits (0.0 invokes default behavior); blastn/megablast 50, tblastx 0, all others 25 [Integer]; default = 0; -R PSI-TBLASTN checkpoint file [File In] Optional; -n MegaBlast search [T/F]; default = F; -L Location on query sequence [String] Optional; -A Multiple Hits window size, default if zero (blastn/megablast 0, all others 40 [Integer]; default = 0; -w Frame shift penalty (OOF algorithm for blastx) [Integer]; default = 0; -t Length of the largest intron allowed in tblastn for linking HSPs (0 disables linking) [Integer]; default = 0.
- 40 **[0185.1.1.1]** Results of high quality are reached by using the algorithm of Needleman and Wunsch or Smith and Waterman. Therefore programs based on said algorithms are preferred. Advantageously the comparisons of sequences can be done with

the program PileUp (J. Mol. Evolution., 25, 351 (1987), Higgins et al., CABIOS 5, 151 (1989)) or preferably with the programs "Gap" and "Needle", which are both based on the algorithms of Needleman and Wunsch (J. Mol. Biol. **48**; 443 (1970)), and "BestFit", which is based on the algorithm of Smith and Waterman (Adv. Appl. Math. **2**; 482 (1981)). "Gap" and "BestFit" are part of the GCG software-package (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991); Altschul et al., (Nucleic Acids Res. 25, 3389 (1997)), "Needle" is part of the The European Molecular Biology Open Software Suite (EMBOSS) (Trends in Genetics 16 (6), 276 (2000)). Therefore preferably the calculations to determine the percentages of sequence homology are done with the programs "Gap" or "Needle" over the whole range of the sequences. The following standard adjustments for the comparison of nucleic acid sequences were used for "Needle": matrix: EDNAFULL, Gap_penalty: 10.0, Extend_penalty: 0.5. The following standard adjustments for the comparison of nucleic acid sequences were used for "Gap": gap weight: 50, length weight: 3, average match: 10.000, average mismatch: 0.000.

[0186.1.1.1] For example a sequence, which has 80% homology with sequence SEQ ID NO: 9 at the nucleic acid level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 9 by the above program "Needle" with the above parameter set, has a 80% identity.

[0187.1.1.1] Homology between two polypeptides is understood as meaning the identity of the amino acid sequence over in each case the entire sequence length which is calculated by comparison with the aid of the above program "Needle" using Matrix: EBLOSUM62, Gap_penalty: 8.0, Extend_penalty: 2.0.

[0188.1.1.1] For example a sequence which has a 80% homology with sequence SEQ ID NO: 10 at the protein level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 10 by the above program "Needle" with the above parameter set, has a 80% identity.

[0189.1.1.1] Functional equivalents derived from one of the polypeptides as shown in table II, columns 5 and 7 according to the invention by substitution, insertion or deletion have at least 30%, 35%, 40%, 45% or 50%, preferably at least 55%, 60%, 65% or 70% by preference at least 80%, especially preferably at least 85% or 90%, 91%, 92%, 93% or 94%, very especially preferably at least 95%, 97%, 98% or 99% homology with one of the polypeptides as shown in table II, columns 5 and 7 according to the invention and are distinguished by essentially the same properties as the polypeptide as shown in table II, columns 5 and 7.

[0190.1.1.1] Functional equivalents derived from the nucleic acid sequence as shown in table I, columns 5 and 7 according to the invention by substitution, insertion or deletion have at least 30%, 35%, 40%, 45% or 50%, preferably at least 55%, 60%, 65% or 70% by preference at least 80%, especially preferably at least 85% or 90%, 91%, 92%, 93% or 94%, very especially preferably at least 95%, 97%, 98% or 99%

homology with one of the polypeptides as shown in table II, columns 5 and 7 according to the invention and encode polypeptides having essentially the same properties as the polypeptide as shown in table II, columns 5 and 7.

[0191.1.1.1] “Essentially the same properties” of a functional equivalent is above all understood as meaning that the functional equivalent has above mentioned activity, by for example expression either in the cytosol or in an organelle such as a plastid or mitochondria or both, preferably in plastids while increasing the amount of protein, activity or function of said functional equivalent in an organism, e.g. a microorganism, a plant or plant or animal tissue, plant or animal cells or a part of the same.

[0192.1.1.1] A nucleic acid molecule encoding an homologous to a protein sequence of table II, columns 5 and 7 can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of the nucleic acid molecule of the present invention, in particular of table I, columns 5 and 7 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into the encoding sequences of table I, columns 5 and 7 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

[0193.1.1.1] Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0194.1.1.1] Thus, a predicted nonessential amino acid residue in a polypeptide of the invention or a polypeptide used in the process of the invention is preferably replaced with another amino acid residue from the same family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a coding sequence of a nucleic acid molecule of the invention or used in the process of the invention, such as by saturation mutagenesis, and the resultant mutants can be screened for activity described herein to identify mutants that retain or even have increased above mentioned activity, e.g. conferring an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, plant or part thereof .

[0195.1.1.1] Following mutagenesis of one of the sequences of shown herein, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using, for example, assays described herein (see Examples).

5 **[0196.1.1.1]** A high homology of the nucleic acid molecule used in the process according to the invention can be found for database entries by Gap search.

[0197.1.1.1] Homologues of the nucleic acid sequences used, with the sequence shown in table I, columns 5 and 7, comprise also allelic variants with at least approximately 30%, 35%, 40% or 45% homology, by preference at least approximately 50%, 60% or 70%, more preferably at least approximately 90%, 91%, 92%, 93%, 94% or
10 95% and even more preferably at least approximately 96%, 97%, 98%, 99% or more homology with one of the nucleotide sequences shown or the abovementioned derived nucleic acid sequences or their homologues, derivatives or analogues or parts of these. Allelic variants encompass in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from the sequences shown, preferably
15 from table I, columns 5 and 7, or from the derived nucleic acid sequences, the intention being, however, that the enzyme activity or the biological activity of the resulting proteins synthesized is advantageously retained or increased.

[0198.1.1.1] In one embodiment of the present invention, the nucleic acid molecule of the invention or used in the process of the invention comprises the sequences
20 shown in any of the table I, columns 5 and 7. It is preferred that the nucleic acid molecule comprises as little as possible other nucleotides not shown in any one of table I, columns 5 and 7. In one embodiment, the nucleic acid molecule comprises less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50 or 40 further nucleotides. In a further embodiment, the nucleic acid molecule comprises less than 30, 20 or 10 further nucleotides. In one embodiment, the nucleic acid molecule use in the process of the invention
25 is identical to the sequences shown in table I, columns 5 and 7.

[0199.1.1.1] Also preferred is that the nucleic acid molecule used in the process of the invention encodes a polypeptide comprising the sequence shown in table II, columns 5 and 7. In one embodiment, the nucleic acid molecule encodes less than 150,
30 130, 100, 80, 60, 50, 40 or 30 further amino acids. In a further embodiment, the encoded polypeptide comprises less than 20, 15, 10, 9, 8, 7, 6 or 5 further amino acids. In one embodiment used in the inventive process, the encoded polypeptide is identical to the sequences shown in table II, columns 5 and 7.

[0200.1.1.1] In one embodiment, the nucleic acid molecule of the invention or used
35 in the process encodes a polypeptide comprising the sequence shown in table II, columns 5 and 7 comprises less than 100 further nucleotides. In a further embodiment, said nucleic acid molecule comprises less than 30 further nucleotides. In one embodiment, the nucleic acid molecule used in the process is identical to a coding sequence of the sequences shown in table I, columns 5 and 7.

[0201.1.1.1] Polypeptides (= proteins), which still have the essential biological or enzymatic activity of the polypeptide of the present invention conferring an increased biotic stress resistance and preferably a further yield related trait as compared to a corresponding non-transformed wild type plant cell, plant or part thereof i.e. whose activity is essentially not reduced, are polypeptides with at least 10% or 20%, by preference 30% or 40%, especially preferably 50% or 60%, very especially preferably 80% or 90 or more of the wild type biological activity or enzyme activity, advantageously, the activity is essentially not reduced in comparison with the activity of a polypeptide shown in table II, columns 5 and 7 expressed under identical conditions.

[0202.1.1.1] Homologues of table I, columns 5 and 7 or of the derived sequences of table II, columns 5 and 7 also mean truncated sequences, cDNA, single-stranded DNA or RNA of the coding and noncoding DNA sequence. Homologues of said sequences are also understood as meaning derivatives, which comprise noncoding regions such as, for example, UTRs, terminators, enhancers or promoter variants. The promoters upstream of the nucleotide sequences stated can be modified by one or more nucleotide substitution(s), insertion(s) and/or deletion(s) without, however, interfering with the functionality or activity either of the promoters, the open reading frame (= ORF) or with the 3'-regulatory region such as terminators or other 3' regulatory regions, which are far away from the ORF. It is furthermore possible that the activity of the promoters is increased by modification of their sequence, or that they are replaced completely by more active promoters, even promoters from heterologous organisms. Appropriate promoters are known to the person skilled in the art and are mentioned herein below.

[0203.1.1.1] In addition to the nucleic acid molecules encoding the BSRP described above, another aspect of the invention pertains to negative regulators of the activity of a nucleic acid molecules selected from the group according to table I, column 5 and/or 7, preferably column 7. Antisense polynucleotides thereto are thought to inhibit the downregulating activity of those negative regulators by specifically binding the target polynucleotide and interfering with transcription, splicing, transport, translation, and/or stability of the target polynucleotide. Methods are described in the prior art for targeting the antisense polynucleotide to the chromosomal DNA, to a primary RNA transcript, or to a processed mRNA. Preferably, the target regions include splice sites, translation initiation codons, translation termination codons, and other sequences within the open reading frame.

[0204.1.1.1] The term "antisense," for the purposes of the invention, refers to a nucleic acid comprising a polynucleotide that is sufficiently complementary to all or a portion of a gene, primary transcript, or processed mRNA, so as to interfere with expression of the endogenous gene. "Complementary" polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two

polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other. The term "antisense nucleic acid" includes single stranded RNA as well as double-stranded DNA expression cassettes that can be transcribed to produce an antisense RNA. "Active" antisense nucleic acids are antisense RNA molecules that are capable of selectively hybridizing with a negative regulator of the activity of a nucleic acid molecules encoding a polypeptide having at least 80% sequence identity with the polypeptide selected from the group according to table II, column 5 and/or 7, preferably column 7..

- 10 The antisense nucleic acid can be complementary to an entire negative regulator strand, or to only a portion thereof. In an embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a BSRP. The term "noncoding region" refers to 5' and 3' sequences that flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions). The antisense nucleic acid molecule can be complementary to only a portion of the noncoding region of BSRP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of BSRP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. Typically, the antisense molecules of the present invention comprise an RNA having 60-100% sequence identity with at least 14 consecutive nucleotides of a noncoding region of one of the nucleic acid of table I. Preferably, the sequence identity will be at least 70%, more preferably at least 75%, 80%, 85%, 90%, 95%, 98% and most preferably 99%.

- An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxycarboxymethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-

oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0205.1.1.1] In yet another embodiment, the antisense nucleic acid molecule of the invention is an alpha-anomeric nucleic acid molecule. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gaultier et al., 1987, Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., 1987, Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

[0206.1.1.1] The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic (including plant) promoter are preferred.

[0207.1.1.1] As an alternative to antisense polynucleotides, ribozymes, sense polynucleotides, or double stranded RNA (dsRNA) can be used to reduce expression of a BSRP polypeptide. By "ribozyme" is meant a catalytic RNA-based enzyme with ribonuclease activity which is capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which it has a complementary region. Ribozymes (e.g., hammerhead ribozymes described in Haselhoff and Gerlach, 1988, Nature 334:585-591) can be used to catalytically cleave BSRP mRNA transcripts to thereby inhibit translation of BSRP mRNA. A ribozyme having specificity for a BSRP-encoding nucleic acid can be designed based upon the nucleotide sequence of a BSRP cDNA, as disclosed herein or on the basis of a heterologous sequence to be isolated according to methods taught in this invention. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a BSRP-encoding mRNA. See, e.g., U.S. Patent Nos. 4,987,071 and 5,116,742 to Cech et al. Alternatively, BSRP mRNA can be used

to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W., 1993, Science 261:1411-1418. In preferred embodiments, the ribozyme will contain a portion having at least 7, 8, 9, 10, 12, 14, 16, 18 or 20 nucleotides, and more preferably 7 or 8 nucleotides, that have
5 100% complementarity to a portion of the target RNA. Methods for making ribozymes are known to those skilled in the art. See, e.g., U.S. Patent Nos. 6,025,167; 5,773,260; and 5,496,698.

The term "dsRNA," as used herein, refers to RNA hybrids comprising two strands of RNA. The dsRNAs can be linear or circular in structure. In a preferred embodiment,
10 dsRNA is specific for a polynucleotide encoding either the polypeptide according to table II or a polypeptide having at least 70% sequence identity with a polypeptide according to table II. The hybridizing RNAs may be substantially or completely complementary. By "substantially complementary," is meant that when the two hybridizing RNAs are optimally aligned using the BLAST program as described above, the hybrid-
15 izing portions are at least 95% complementary. Preferably, the dsRNA will be at least 100 base pairs in length. Typically, the hybridizing RNAs will be of identical length with no over hanging 5' or 3' ends and no gaps. However, dsRNAs having 5' or 3' overhangs of up to 100 nucleotides may be used in the methods of the invention.

The dsRNA may comprise ribonucleotides or ribonucleotide analogs, such as 2'-O-methyl ribosyl residues, or combinations thereof. See, e.g., U.S. Patent Nos.
20 4,130,641 and 4,024,222. A dsRNA polyribonucleosinic acid:polyribocytidylic acid is described in U.S. patent 4,283,393. Methods for making and using dsRNA are known in the art. One method comprises the simultaneous transcription of two complementary DNA strands, either *in vivo*, or in a single *in vitro* reaction mixture. See, e.g., U.S. Pat-
25 ent No. 5,795,715. In one embodiment, dsRNA can be introduced into a plant or plant cell directly by standard transformation procedures. Alternatively, dsRNA can be expressed in a plant cell by transcribing two complementary RNAs.

[0208.1.1.1] Other methods for the inhibition of endogenous gene expression, such as triple helix formation (Moser et al., 1987, Science 238:645-650 and Cooney et al.,
30 1988, Science 241:456-459) and cosuppression (Napoli et al., 1990, The Plant Cell 2:279-289) are known in the art. Partial and full-length cDNAs have been used for the cosuppression of endogenous plant genes. See, e.g., U.S. Patent Nos. 4,801,340, 5,034,323, 5,231,020, and 5,283,184; Van der Kroll et al., 1990, The Plant Cell 2:291-299; Smith et al., 1990, Mol. Gen. Genetics 224:477-481 and Napoli et al., 1990, The
35 Plant Cell 2:279-289.

For sense suppression, it is believed that introduction of a sense polynucleotide blocks transcription of the corresponding target gene. The sense polynucleotide will have at least 65% sequence identity with the target plant gene or RNA. Preferably, the percent identity is at least 80%, 90%, 95% or more. The introduced sense polynucleotide need
40 not be full length relative to the target gene or transcript. Preferably, the sense polynucleotide will have at least 65% sequence identity with at least 100 consecutive nucleo-

tides of one of the nucleic acids as depicted in Table I. The regions of identity can comprise introns and and/or exons and untranslated regions. The introduced sense polynucleotide may be present in the plant cell transiently, or may be stably integrated into a plant chromosome or extrachromosomal replicon.

- 5 **[0209.1.1.1]** Further, object of the invention is an expression vector comprising a nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of:
- a) a nucleic acid molecule encoding the polypeptide shown in column 5 or 7 of Table II;
 - 10 b) a nucleic acid molecule shown in column 5 or 7 of Table I;
 - c) a nucleic acid molecule, which, as a result of the degeneracy of the genetic code, can be derived from a polypeptide sequence depicted in column 5 or 7 of Table II and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
 - 15 d) a nucleic acid molecule having at least 30 % identity with the nucleic acid molecule sequence of a polynucleotide comprising the nucleic acid molecule shown in column 5 or 7 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
 - 20 e) a nucleic acid molecule encoding a polypeptide having at least 30 % identity with the amino acid sequence of the polypeptide encoded by the nucleic acid molecule of (a) to (c) and having the activity represented by a polypeptide comprising a polypeptide as depicted in column 5 of Table I and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
 - 25 f) nucleic acid molecule which hybridizes with a nucleic acid molecule of (a) to (c) under stringent hybridization conditions and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
 - 30 g) a nucleic acid molecule encoding a polypeptide which can be isolated with the aid of monoclonal or polyclonal antibodies made against a polypeptide encoded by one of the nucleic acid molecules of (a) to (e) and having the activity represented by the nucleic acid molecule comprising a polynucleotide as depicted in column 5 of Table I;
 - 35

- h) a nucleic acid molecule encoding a polypeptide comprising the consensus sequence or one or more polypeptide motifs as shown in column 7 of Table IV and preferably having the activity represented by polypeptide comprising a polypeptide as depicted in column 5 of Table II or IV;
- 5 h) a nucleic acid molecule encoding a polypeptide having the activity represented by a protein as depicted in column 5 of Table II and confers an increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 10 i) nucleic acid molecule which comprises a polynucleotide, which is obtained by amplifying a cDNA library or a genomic library using the primers in column 7 of Table III and preferably having the activity represented by a polypeptide comprising a polypeptide as depicted in column 5 of Table II or IV;
- 15 and
- j) a nucleic acid molecule which is obtainable by screening a suitable nucleic acid library under stringent hybridization conditions with a probe comprising a complementary sequence of a nucleic acid molecule of (a) or (b) or with a fragment thereof, having at least 15 nt, preferably 20 nt, 30 nt, 50
20 nt, 100 nt, 200 nt or 500 nt of a nucleic acid molecule complementary to a nucleic acid molecule sequence characterized in (a) to (e) and encoding a polypeptide having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II,

whereby the nucleic acid molecule according to (a) to (j) is at least in one or more nucleotides, preferably different from the sequence depicted in column 5 or 7 of Table I A
25 and preferably which encodes a protein which preferably differs at least in one or more amino acids from the protein sequences depicted in column 5 or 7 of Table II A.

[0210.1.1.1] The invention further provides an isolated recombinant expression vector comprising a stress related protein encoding nucleic acid as described above,
30 wherein expression of the vector or stress related protein encoding nucleic acid, respectively in a host cell results in increased tolerance and/or resistance to stress, preferably plant disease, preferably caused by pathogenic fungi as compared to the corresponding non-transformed wild type of the host cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it
35 has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Further types of vectors can be linearized nucleic acid sequences, such as transposons, which are pieces of DNA which can copy and insert themselves. There
40 have been 2 types of transposons found: simple transposons, known as Insertion Se-

quences and composite transposons, which can have several genes as well as the genes that are required for transposition.

Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal
5 mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in
10 recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent func-
15 tions.

[0211.1.1.1] A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells and operably linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from *Agrobacterium tumefaciens* T-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984 EMBO J. 3:835) or functional equivalents thereof
20 but also all other terminators functionally active in plants are suitable.

As plant gene expression is very often not limited on transcriptional levels, a plant expression cassette preferably contains other operably linked sequences like translational
25 enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the protein per RNA ratio (Gallie et al., 1987 Nucl. Acids Research 15:8693-8711).

[0212.1.1.1] Plant gene expression has to be operably linked to an appropriate promoter conferring gene expression in a timely, cell or tissue specific manner. Preferred
30 are promoters driving constitutive expression (Benfey et al., 1989 EMBO J. 8:2195-2202) like those derived from plant viruses like the 35S CaMV (Franck et al., 1980 Cell 21:285-294), the 19S CaMV (see also U.S. Patent No. 5352605 and PCT Application No. WO 8402913) or plant promoters like those from Rubisco small subunit described in U.S. Patent No. 4,962,028.

[0213.1.1.1] Additional advantageous regulatory sequences are, for example, included in the plant promoters such as CaMV/35S [Franck et al., Cell 21 (1980) 285 - 294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, LEB4, nos or in the ubiquitin, napin or phaseolin promoter. Also advantageous in this connection are inducible promoters such as the promoters described in EP-A-0
40 388 186 (benzyl sulfonamide inducible), Plant J. 2, 1992: 397 - 404 (Gatz et al., Tetra-

cyclin inducible), EP-A-0 335 528 (abscisic acid inducible) or WO 93/21334 (ethanol or cyclohexenol inducible). Additional useful plant promoters are the cytosolic FBPase promoter or ST-LSI promoter of the potato (Stockhaus et al., EMBO J. 8, 1989, 2445), the phosphorybosyl phyrophoshate amido transferase promoter of Glycine max (gene bank accession No. U87999) or the noden specific promoter described in EP-A-0 249 676. Additional particularly advantageous promoters are seed specific promoters which can be used for monokotyledones or dikotyledones and are described in US 5,608,152 (napin promoter from rapeseed), WO 98/45461 (phaseolin promoter from Arobidopsis), US 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica) and Baeumlein et al., Plant J., 2, 2, 1992: 233-239 (LEB4 promoter from leguminosa). Said promoters are useful in dikotyledones. The following promoters are useful for example in monokotyledones lpt-2- or lpt-1- promoter from barley (WO 95/15389 and WO 95/23230) or hordein promoter from barley. Other useful promoters are described in WO 99/16890.

It is possible in principle to use all natural promoters with their regulatory sequences like those mentioned above for the novel process. It is also possible and advantageous in addition to use synthetic promoters.

[0214.1.1.1] The gene construct may also comprise further genes which are to be inserted into the organisms and which are for example involved in stress resistance increase. These genes can be heterologous or homologous in origin. The inserted genes may have their own promoter or else be under the control of same promoter as the sequences of the nucleic acid of table I or their homologs.

The gene construct advantageously comprises, for expression of the other genes present, additionally 3' and/or 5' terminal regulatory sequences to enhance expression, which are selected for optimal expression depending on the selected host organism and gene or genes.

[0215.1.1.1] These regulatory sequences are intended to make specific expression of the genes and protein expression possible as mentioned above. This may mean, depending on the host organism, for example that the gene is expressed or overexpressed only after induction, or that it is immediately expressed and/or overexpressed.

The regulatory sequences or factors may moreover preferably have a beneficial effect on expression of the introduced genes, and thus increase it. It is possible in this way for the regulatory elements to be enhanced advantageously at the transcription level by using strong transcription signals such as promoters and/or enhancers. However, in addition, it is also possible to enhance translation by, for example, improving the stability of the mRNA.

[0216.1.1.1] Other preferred sequences for use in plant gene expression cassettes are targeting-sequences necessary to direct the gene product in its appropriate cell compartment (for review see Kermode, 1996 Crit. Rev. Plant Sci. 15(4):285-423 and references cited therein) such as the vacuole, the nucleus, all types of plastids like

amyloplasts, chloroplasts, chromoplasts, the extracellular space, mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells.

- Plant gene expression can also be facilitated via an inducible promoter (for review see Gatz, 1997 Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if gene expression is wanted to occur in a time specific manner.

[0217.1.1.1] Table 1 lists several examples of promoters that may be used to regulate transcription of the stress related protein nucleic acid coding sequences.

Tab. 1: Examples of tissue-specific and stress-inducible promoters in plants

Expression	Reference
Cor78- Cold, drought, salt, ABA, wounding-inducible	Ishitani, <i>et al.</i> , Plant Cell 9:1935-1949 (1997). Yamaguchi-Shinozaki and Shinozaki, Plant Cell 6:251-264 (1994).
Rci2A - Cold, dehydration-inducible	Capel <i>et al.</i> , Plant Physiol 115:569-576 (1997)
Rd22 - Drought, salt	Yamaguchi-Shinozaki and Shinozaki, Mol Gen Genet 238:17-25 (1993).
Cor15A - Cold, dehydration, ABA	Baker <i>et al.</i> , Plant Mol. Biol. 24:701-713 (1994).
GH3- Auxin inducible	Liu <i>et al.</i> , Plant Cell 6:645-657 (1994)
ARSK1-Root, salt inducible	Hwang and Goodman, Plant J 8:37-43 (1995).
PtxA - Root, salt inducible	GenBank accession X67427
SbHRGP3 - Root specific	Ahn <i>et al.</i> , Plant Cell 8:1477-1490 (1998).
KST1 - Guard cell specific	Plesch <i>et al.</i> , Plant Journal. 28(4):455-64, (2001)
KAT1 - Guard cell specific	Plesch <i>et al.</i> , Gene 249:83-89 (2000) Nakamura <i>et al.</i> , Plant Physiol. 109:371-374 (1995)
salicylic acid inducible	PCT Application No. WO 95/19443
tetracycline inducible	Gatz et al. Plant J. 2:397-404 (1992)
Ethanol inducible	PCT Application No. WO 93/21334
pathogen inducible PRP1	Ward et al., 1993 Plant. Mol. Biol. 22:361-366
heat inducible hsp80	U.S. Patent No. 5187267
cold inducible alpha-amylase	PCT Application No. WO 96/12814

Expression	Reference
Wound-inducible pinII	European Patent No. 375091
RD29A - salt-inducible	Yamaguchi-Shinozaki et al. (1993) Mol. Gen. Genet. 236:331-340
plastid-specific viral RNA-polymerase	PCT Application No. WO 95/16783 and. WO 97/06250

[0218.1.1.1] Other promoters, e.g. superpromotor (Ni et al., Plant Journal 7, 1995: 661-676), Ubiquitin promotor (Callis et al., J. Biol. Chem., 1990, 265: 12486-12493; US 5,510,474; US 6,020,190; Kawalleck et al., Plant. Molecular Biology, 1993, 21: 673-684) or 34S promotor (GenBank Accession numbers M59930 and X16673) were similar useful for the present invention and are known to a person skilled in the art.

Developmental stage-preferred promoters are preferentially expressed at certain stages of development. Tissue and organ preferred promoters include those that are preferentially expressed in certain tissues or organs, such as leaves, roots, seeds, or xylem. Examples of tissue preferred and organ preferred promoters include, but are not limited to fruit-preferred, ovule-preferred, male tissue-preferred, seed-preferred, integument-preferred, tuber-preferred, stalk-preferred, pericarp-preferred, and leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, a petal-preferred, sepal-preferred, pedicel-preferred, silique-preferred, stem-preferred, root-preferred promoters, and the like. Seed preferred promoters are preferentially expressed during seed development and/or germination. For example, seed preferred promoters can be embryo-preferred, endosperm preferred, and seed coat-preferred. See Thompson et al., 1989, BioEssays 10:108. Examples of seed preferred promoters include, but are not limited to, cellulose synthase (celA), Cim1, gamma-zein, globulin-1, maize 19 kD zein (cZ19B1), and the like.

Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β -conglycin promoter, the napin promoter, the soybean lectin promoter, the maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2 and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[0219.1.1.1] Additional flexibility in controlling heterologous gene expression in plants may be obtained by using DNA binding domains and response elements from heterologous sources (*i.e.*, DNA binding domains from non-plant sources). An example of such a heterologous DNA binding domain is the LexA DNA binding domain (Brent and Ptashne, 1985, Cell 43:729-736).

[0220.1.1.1] The invention further provides a recombinant expression vector comprising a BSRP DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to a BSRP mRNA. Regulatory sequences operatively linked to a nucleic acid molecule cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types. For instance, viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus wherein antisense nucleic acids are produced under the control of a high efficiency regulatory region. The activity of the regulatory region can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. et al., 1986, Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Vol. 1(1), and Mol et al., 1990, FEBS Letters 268:427-430.

[0221.1.1.1] Another aspect of the invention pertains to isolated BSRP, and biologically active portions thereof. An "isolated" or "purified" polypeptide or biologically active portion thereof is free of some of the cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of BSRP in which the polypeptide is separated from some of the cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a BSRP having less than about 30% (by dry weight) of non-BSRP material (also referred to herein as a "contaminating polypeptide"), more preferably less than about 20% of non-BSRP material, still more preferably less than about 10% of non-BSRP material, and most preferably less than about 5% non-BSRP material.

[0222.1.1.1] When the BSRP or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the polypeptide preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of BSRP in which the polypeptide is separated from chemical precursors or other chemicals that are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of a BSRP having less than about 30% (by dry weight) of chemical precursors or non-BSRP chemicals, more preferably less than about 20% chemical precursors or non-BSRP chemicals, still more preferably less than about 10% chemical precursors or non-BSRP chemicals, and most preferably less than about 5% chemical precursors or

non-BSRP chemicals. In preferred embodiments, isolated polypeptides, or biologically active portions thereof, lack contaminating polypeptides from the same organism from which the BSRP is derived. Typically, such polypeptides are produced by recombinant expression of, for example, a *Saccharomyces cerevisiae*, *E.coli* or *Brassica napus*,
5 *Glycine max*, *Zea mays* or *Oryza sativa* BSRP in plants other than *Saccharomyces cerevisiae*, *E.coli*, or microorganisms such as *C. glutamicum*, ciliates, algae or fungi.

[0223.1.1.1] The nucleic acid molecules, polypeptides, polypeptide homologs, fusion polypeptides, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *Saccharomyces cerevisiae*, *E.coli* or
10 *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* and related organisms; mapping of genomes of organisms related to *Saccharomyces cerevisiae*, *E.coli*; identification and localization of *Saccharomyces cerevisiae*, *E.coli* or *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* sequences of interest; evolutionary studies; determination of BSRP regions required for function; modulation of a BSRP activity; modulation
15 of the metabolism of one or more cell functions; modulation of the transmembrane transport of one or more compounds; modulation of stress resistance; and modulation of expression of BSRP nucleic acids.

[0224.1.1.1] The BSRP nucleic acid molecules of the invention are also useful for evolutionary and polypeptide structural studies. The metabolic and transport processes in which the molecules of the invention participate are utilized by a wide variety
20 of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the polypeptide that are essential for the functioning of the enzyme. This type of determination is of
25 value for polypeptide engineering studies and may give an indication of what the polypeptide can tolerate in terms of mutagenesis without losing function.

[0225.1.1.1] Manipulation of the BSRP nucleic acid molecules of the invention may
30 result in the production of BSRP having functional differences from the wild-type BSRP. These polypeptides may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

There are a number of mechanisms by which the alteration of a BSRP of the invention may directly affect stress response and/or stress tolerance. In the case of plants expressing BSRP, increased transport can lead to improved salt and/or solute partitioning
35 within the plant tissue and organs. By either increasing the number or the activity of transporter molecules which export ionic molecules from the cell, it may be possible to affect the salt and cold tolerance of the cell.

[0226.1.1.1] The effect of the genetic modification in plants, on stress tolerance can
40 be assessed by growing the modified plant under less than suitable conditions and

- then analyzing the growth characteristics and/or metabolism of the plant. Such analysis techniques are well known to one skilled in the art, and include dry weight, wet weight, polypeptide synthesis, carbohydrate synthesis, lipid synthesis, evapotranspiration rates, general plant and/or crop yield, flowering, reproduction, seed setting, root growth, respiration rates, photosynthesis rates, etc. (Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al., 1993 Biotechnology, vol. 3, Chapter III: Product recovery and purification, page 469-714, VCH: Weinheim; Belter, P.A. et al., 1988, Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S., 1992, Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D., 1988, Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J., 1989, Separation and purification techniques in biotechnology, Noyes Publications).
- For example, yeast expression vectors comprising the nucleic acids disclosed herein, or fragments thereof, can be constructed and transformed into *Saccharomyces cerevisiae* using standard protocols. The resulting transgenic cells can then be assayed for fail or alteration of their tolerance to biotic stress, preferably caused by plant disease, preferably by pathogenic fungi or drought, salt, and cold stress. Similarly, plant expression vectors comprising the nucleic acids disclosed herein, or fragments thereof, can be constructed and transformed into an appropriate plant cell such as *Arabidopsis*, soy, rape, maize, cotton, rice, wheat, *Medicago truncatula*, etc., using standard protocols. The resulting transgenic cells and/or plants derived therefrom can then be assayed for fail or alteration of their tolerance to biotic stress, preferably caused by plant disease, preferably by pathogenic or drought, salt, cold stress .

- [0227.1.1.1]** The engineering of one or more genes according to table I and coding for the BSRP of table II of the invention may also result in BSRP having altered activities which indirectly impact the stress response and/or stress tolerance of algae, plants, ciliates, or fungi, or other microorganisms like *C. glutamicum*.
- [0228.1.1.1]** Additionally, the sequences disclosed herein, or fragments thereof, can be used to generate knockout mutations in the genomes of various organisms, such as bacteria, mammalian cells, yeast cells, and plant cells (Girke, T., 1998, The Plant Journal 15:39-48). The resultant knockout cells can then be evaluated for their ability or capacity to tolerate various stress conditions, their response to various stress conditions, and the effect on the phenotype and/or genotype of the mutation. For other methods of gene inactivation, see U.S. Patent No. 6,004,804 "Non-Chimeric Mutational Vectors" and Puttaraju et al., 1999, Spliceosome-mediated RNA *trans*-splicing as a tool for gene therapy, Nature Biotechnology 17:246-252.

- The aforementioned mutagenesis strategies for BSRP resulting in increased stress resistance are not meant to be limiting; variations on these strategies will be readily apparent to one skilled in the art. Using such strategies, and incorporating the mecha-

nisms disclosed herein, the nucleic acid and polypeptide molecules of the invention may be utilized to generate algae, ciliates, plants, fungi, or other microorganisms like *C. glutamicum* expressing mutated BSRP nucleic acid and polypeptide molecules such that the stress tolerance is improved.

- 5 **[0228.2.1.1]** The present invention also provides antibodies that specifically bind to a BSRP, or a portion thereof, as encoded by a nucleic acid described herein. Antibodies can be made by many well-known methods (See, e.g. Harlow and Lane, "Antibodies; A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1988)). Briefly, purified antigen can be injected into an animal in an amount and in
10 intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells can then fused with an immortal cell line and screened for antibody secretion. The antibodies can be used to screen nucleic acid clone libraries for cells secreting the antigen. Those positive clones can then be sequenced. See, for example, Kelly et al., 1992, Bio/Technology
15 10:163-167; Bebbington et al., 1992, Bio/Technology 10:169-175.

The phrases "selectively binds" and "specifically binds" with the polypeptide refer to a binding reaction that is determinative of the presence of the polypeptide in a heterogeneous population of polypeptides and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bound to a particular polypeptide do not
20 bind in a significant amount to other polypeptides present in the sample. Selective binding of an antibody under such conditions may require an antibody that is selected for its specificity for a particular polypeptide. A variety of immunoassay formats may be used to select antibodies that selectively bind with a particular polypeptide. For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a polypeptide. See Harlow and Lane, "Antibodies, A Laboratory Manual," Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that could be used to determine selective binding.
25

In some instances, it is desirable to prepare monoclonal antibodies from various hosts.
30 A description of techniques for preparing such monoclonal antibodies may be found in Stites et al., eds., "Basic and Clinical Immunology," (Lange Medical Publications, Los Altos, Calif., Fourth Edition) and references cited therein, and in Harlow and Lane, "Antibodies, A Laboratory Manual," Cold Spring Harbor Publications, New York, (1988).

[0229.1.1.1] Gene expression in plants is regulated by the interaction of protein
35 transcription factors with specific nucleotide sequences within the regulatory region of a gene. One example of transcription factors are polypeptides that contain zinc finger (ZF) motifs. Each ZF module is approximately 30 amino acids long folded around a zinc ion. The DNA recognition domain of a ZF protein is a α -helical structure that inserts into the major groove of the DNA double helix. The module contains three amino
40 acids that bind to the DNA with each amino acid contacting a single base pair in the target DNA sequence. ZF motifs are arranged in a modular repeating fashion to form a

set of fingers that recognize a contiguous DNA sequence. For example, a three-fingered ZF motif will recognize 9 bp of DNA. Hundreds of proteins have been shown to contain ZF motifs with between 2 and 37 ZF modules in each protein (Isalan M, et al., 1998 Biochemistry 37(35):12026-33; Moore M, et al., 2001 Proc. Natl. Acad. Sci. USA 98(4):1432-1436 and 1437-1441; US patents US 6007988 and US 6013453).

The regulatory region of a plant gene contains many short DNA sequences (cis-acting elements) that serve as recognition domains for transcription factors, including ZF proteins. Similar recognition domains in different genes allow the coordinate expression of several genes encoding enzymes in a metabolic pathway by common transcription factors. Variation in the recognition domains among members of a gene family facilitates differences in gene expression within the same gene family, for example, among tissues and stages of development and in response to environmental conditions.

Typical ZF proteins contain not only a DNA recognition domain but also a functional domain that enables the ZF protein to activate or repress transcription of a specific gene. Experimentally, an activation domain has been used to activate transcription of the target gene (US patent 5789538 and patent application WO9519431), but it is also possible to link a transcription repressor domain to the ZF and thereby inhibit transcription (patent applications WO00/47754 and WO2001002019). It has been reported that an enzymatic function such as nucleic acid cleavage can be linked to the ZF (patent application WO00/20622)

[0230.1.1.1] The invention provides a method that allows one skilled in the art to isolate the regulatory region of one or more stress related protein encoding genes from the genome of a plant cell and to design zinc finger transcription factors linked to a functional domain that will interact with the regulatory region of the gene. The interaction of the zinc finger protein with the plant gene can be designed in such a manner as to alter expression of the gene and preferably thereby to confer increased biotic stress resistance and preferably a further yield related trait.

[0231.1.1.1] In particular, the invention provides a method of producing a transgenic plant with a stress related protein coding nucleic acid, wherein expression of the nucleic acid(s) in the plant results in increased tolerance to environmental stress as compared to a wild type plant comprising: (a) transforming a plant cell with an expression vector comprising a stress related protein encoding nucleic acid, and (b) generating from the plant cell a transgenic plant with an increased tolerance to environmental stress as compared to a wild type plant. For such plant transformation, binary vectors such as pBinAR can be used (Höfgen and Willmitzer, 1990 Plant Science 66:221-230). Moreover suitable binary vectors are for example pBIN19, pBI101, pGPTV or pPZP (Hajukiewicz, P. et al., 1994, Plant Mol. Biol., 25: 989-994).

Construction of the binary vectors can be performed by ligation of the cDNA into the T-DNA. 5' to the cDNA a plant promoter activates transcription of the cDNA. A polyadenylation sequence is located 3' to the cDNA. Tissue-specific expression can be

achieved by using a tissue specific promoter as listed above. Also, any other promoter element can be used. For constitutive expression within the whole plant, the CaMV 35S promoter can be used. The expressed protein can be targeted to a cellular compartment using a signal peptide, for example for plastids, mitochondria or endoplasmic reticulum (Kermode, 1996 Crit. Rev. Plant Sci. 4(15):285-423). The signal peptide is cloned 5' in frame to the cDNA to archive subcellular localization of the fusion protein. Additionally, promoters that are responsive to abiotic stresses can be used with, such as the Arabidopsis promoter RD29A. One skilled in the art will recognize that the promoter used should be operatively linked to the nucleic acid such that the promoter causes transcription of the nucleic acid which results in the synthesis of a mRNA which encodes a polypeptide.

[0232.1.1.1] Alternate methods of transfection include the direct transfer of DNA into developing flowers via electroporation or Agrobacterium mediated gene transfer. Agrobacterium mediated plant transformation can be performed using for example the GV3101(pMP90) (Koncz and Schell, 1986 Mol. Gen. Genet. 204:383-396) or LBA4404 (Ooms et al., Plasmid, 1982, 7: 15-29; Hoekema et al., Nature, 1983, 303: 179-180) Agrobacterium tumefaciens strain. Transformation can be performed by standard transformation and regeneration techniques (Deblaere et al., 1994 Nucl. Acids. Res. 13:4777-4788; Gelvin and Schilperoort, Plant Molecular Biology Manual, 2nd Ed. - Dordrecht : Kluwer Academic Publ., 1995. - in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, B R and Thompson, J E, Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993. - 360 S., ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989 Plant Cell Reports 8:238-242; De Block et al., 1989 Plant Physiol. 91:694-701). Use of antibiotics for Agrobacterium and plant selection depends on the binary vector and the Agrobacterium strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. Agrobacterium mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994 Plant Cell Report 13:282-285. Additionally, transformation of soybean can be performed using for example a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent No. 0397 687, U.S. Patent No. 5,376,543 or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbide fiber technique (see, for example, Freeling and Walbot "The maize handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387 and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

[0233.1.1.1] Growing the modified plants under stress conditions and then screening and analyzing the growth characteristics and/or metabolic activity assess the effect of the genetic modification in plants on increased biotic stress resistance and prefera-

bly a further yield related trait. Such analysis techniques are well known to one skilled in the art. They include next to screening (Römpp Lexikon Biotechnologie, Stuttgart/New York: Georg Thieme Verlag 1992, "screening" p. 701) dry weight, wet weight, protein synthesis, carbohydrate synthesis, lipid synthesis, evapotranspiration rates, general plant and/or crop yield, flowering, reproduction, seed setting, root growth, respiration rates, photosynthesis rates, etc. (Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al., 1993 Biotechnology, vol. 3, Chapter III: Product recovery and purification, page 469-714, VCH: Weinheim; Belter, P.A. et al., 1988 Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S., 1992 Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D., 1988 Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

- 15 **[0234.1.1.1]** In one embodiment, the present invention relates to a method for the identification of a gene product conferring increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control cell in a cell of an organism for example plant, comprising the following steps:
- 20 a) contacting, e.g. hybridising, some or all nucleic acid molecules of a sample, e.g. cells, tissues, plants or microorganisms or a nucleic acid library, which can contain a candidate gene encoding a gene product conferring increased stress resistance, preferably to plant disease, preferably caused by pathogenic fungi, with a nucleic acid molecule as shown in column 5 or 7 of Table I A or B or a functional homologue thereof;
 - 25 b) identifying the nucleic acid molecules, which hybridize under relaxed stringent conditions with said nucleic acid molecule, in particular to the nucleic acid molecule sequence shown in column 5 or 7 of Table I and, optionally, isolating the full length cDNA clone or complete genomic clone;
 - 30 c) identifying the candidate nucleic acid molecules or a fragment thereof in host cells, preferably in a plant cell
 - d) increasing the expressing of the identified nucleic acid molecules in the host cells for which increased resistance to plant disease, preferably caused by pathogenic fungi as desired
 - e) assaying the level of increased stress resistance of the host cells; and
 - 35 f) identifying the nucleic acid molecule and its gene product which increased expression confers increased stress resistance in the host cell compared to the wild type.

Relaxed hybridisation conditions are: After standard hybridisation procedures washing steps can be performed at low to medium stringency conditions usually with washing conditions of 40°-55°C and salt conditions between 2xSSC and 0,2x SSC with 0,1%

SDS in comparison to stringent washing conditions as e.g. 60° to 68°C with 0,1% SDS. Further examples can be found in the references listed above for the stringent hybridization conditions. Usually washing steps are repeated with increasing stringency and length until a useful signal to noise ratio is detected and depend on many factors as the target, e.g. its purity, GC-content, size etc, the probe, e.g. its length, is it a RNA or a DNA probe, salt conditions, washing or hybridisation temperature, washing or hybridisation time etc.

[0234.2.1.1] In another embodiment, the present invention relates to a method for the identification of a gene product the expression of which confers increased stress resistance in a cell, comprising the following steps:

- a) identifying a nucleic acid molecule in an organism, which is at least 20%, preferably 25%, more preferably 30%, even more preferred are 35%, 40% or 50%, even more preferred are 60%, 70% or 80%, most preferred are 90% or 95% or more homologous to the nucleic acid molecule encoding a protein comprising the polypeptide molecule as shown in column 5 or 7 of Table II or comprising a consensus sequence or a polypeptide motif as shown in column 7 of Table IV or being encoded by a nucleic acid molecule comprising a polynucleotide as shown in column 5 or 7 of Table I or a homologue thereof as described herein, for example via homology search in a data bank;
- b) enhancing the expression of the identified nucleic acid molecules in the host cells;
- c) assaying the level of increased stress resistance, preferably to plant disease, preferably caused by pathogenic fungi in the host cells; and
- d) identifying the host cell, in which the enhanced expression confers increased stress resistance in the host cell compared to a wild type.

[0234.3.1.1] Further, the nucleic acid molecule disclosed herein, in particular the nucleic acid molecule shown column 5 or 7 of Table I A or B, may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related organism or for association mapping. Furthermore natural variation in the genomic regions corresponding to nucleic acids disclosed herein, in particular the nucleic acid molecule shown column 5 or 7 of Table I A or B, or homologous thereof may lead to variation in the activity of the proteins disclosed herein, in particular the proteins comprising polypeptides as shown in column 5 or 7 of Table II A or B or comprising the consensus sequence or the polypeptide motif as shown in column 7 of Table IV, and their homologous and in consequence in natural variation in tolerance and/or resistance to biotic stress and preferably a further yield related trait.

In consequence natural variation eventually also exists in form of more active allelic variants leading already to a relative increase in the tolerance and/or resistance to biotic stress and preferably a further yield related trait. Different variants of the nucleic acids molecule disclosed herein, in particular the nucleic acid comprising the nucleic

acid molecule as shown column 5 or 7 of Table I A or B, which corresponds to different tolerance and/or stress resistance levels can be indentified and used for marker assisted breeding for increased stress resistance, preferably to plant disease, preferably caused by pathogenic fungi.

- 5 **[0234.4.1.1]** Accordingly, the present invention relates to a method for breeding plants for increased stress resistance, preferably to plant disease, preferably caused by pathogenic fungi, comprising
- a) selecting a first plant variety with increased stress resistance, preferably to plant disease, preferably caused by pathogenic fungi based on increased expression of a
- 10 nucleic acid of the invention as disclosed herein, in particular of a nucleic acid molecule comprising a nucleic acid molecule as shown in column 5 or 7 of Table I A or B or a polypeptide comprising a polypeptide as shown in column 5 or 7 of Table II A or B or comprising a consensus sequence or a polypeptide motif as shown in column 7 of Table IV, or a homologue thereof as described herein;
- 15 b) associating the level of tolerance and/or resistance to stress, preferably to plant disease, preferably caused by pathogenic fungi with the expression level or the genomic structure of a gene encoding said polypeptide or said nucleic acid molecule;
- c) crossing the first plant variety with a second plant variety, which significantly differs in its level of tolerance and/or resistance to stress and
- 20 e) identifying, which of the offspring varieties has got increased levels level of tolerance and/or resistance to stress by the expression level of said polypeptide or nucleic acid molecule or the genomic structure of the genes encoding said polypeptide or nucleic acid molecule of the invention.

In one embodiment, the expression level of the gene according to step (b) is increased.

- 25 **[0235.1.1.1]** Yet another embodiment of the invention relates to a process for the identification of a compound conferring increased resistance to biotic stress, preferably pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof in a plant cell, a plant or a part thereof, a plant or a part thereof, comprising the steps:
- 30 a) culturing a plant cell; a plant or a part thereof maintaining a plant expressing the polypeptide as shown in column 5 or 7 of Table II or being encoded by a nucleic acid molecule comprising a polynucleotide as shown in column 5 or 7 of Table I or a homologue thereof as described herein or a polynucleotide encoding said polypeptide and conferring an increased resistance to biotic stress, preferably pathogenic fungi as
- 35 compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof; a non-transformed wild type plant or a part thereof and providing a readout system capable of interacting with the polypeptide under suitable conditions which permit the interaction of the polypeptide with this readout system in the presence of a chemical compound or a sample comprising a plurality of chemical compounds and

capable of providing a detectable signal in response to the binding of a chemical compound to said polypeptide under conditions which permit the expression of said readout system and of the protein as shown in column 5 or 7 of Table II or being encoded by a nucleic acid molecule comprising a polynucleotide as shown in column 5 or 7 of Table I
5 or a homologue thereof as described herein; and

b) identifying if the chemical compound is an effective agonist by detecting the presence or absence or decrease or increase of a signal produced by said readout system.

Said compound may be chemically synthesized or microbiologically produced and/or
10 comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms, e.g. pathogens. Furthermore, said compound(s) may be known in the art but hitherto not known to be capable of activating the polypeptide of the present invention. The reaction mixture may be a cell free extract or may comprise a cell or tissue culture. Suitable set ups for the process for identification of a compound of the
15 invention are known to the person skilled in the art and are, for example, generally described in Alberts et al., Molecular Biology of the Cell, third edition (1994), in particular Chapter 17. The compounds may be, e.g., added to the reaction mixture, culture medium, injected into the cell or sprayed onto the plant.

If a sample containing a compound is identified in the process, then it is either possible
20 to isolate the compound from the original sample identified as containing the compound capable of activating or increasing biotic stress resistance and preferably a further yield related trait as compared to a corresponding non-transformed wild type, or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat
25 the method with the subdivisions of the original sample. Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the said process only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. Preferably, the compound identified according to the described method above or
30 its derivative is further formulated in a form suitable for the application in plant breeding or plant cell and tissue culture.

The compounds which can be tested and identified according to said process may be expression libraries, e.g., cDNA expression libraries, peptides, proteins, nucleic acids,
35 antibodies, small organic compounds, hormones, peptidomimetics, PNAs or the like (Milner, Nature Medicine 1 (1995), 879-880; Hupp, Cell 83 (1995), 237-245; Gibbs, Cell 79 (1994), 193-198 and references cited supra). Said compounds can also be functional derivatives or analogues of known inhibitors or activators. Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art
40 and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic

Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used, for example, according to the methods described above. The cell or tissue that
5 may be employed in the process preferably is a host cell, plant cell or plant tissue of the invention described in the embodiments hereinbefore.

Thus, in a further embodiment the invention relates to a compound obtained or identified according to the method for identifying an agonist of the invention said compound being an antagonist of the polypeptide of the present invention.

10 Accordingly, in one embodiment, the present invention further relates to a compound identified by the method for identifying a compound of the present invention.

[0235.2.1.1] In one embodiment, the invention relates to an antibody specifically recognizing the compound or agonist of the present invention.

[0235.3.1.1] The invention also relates to a diagnostic composition comprising at
15 least one of the aforementioned nucleic acid molecules, antisense nucleic acid molecule, RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, cosuppression molecule, ribozyme, vectors, proteins, antibodies or compounds of the invention and optionally suitable means for detection.

The diagnostic composition of the present invention is suitable for the isolation of
20 mRNA from a cell and contacting the mRNA so obtained with a probe comprising a nucleic acid probe as described above under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the protein in the cell. Further methods of detecting the presence of a protein according to the present invention comprise immunotechniques well known in the art, for example
25 enzyme linked immunoadsorbent assay. Furthermore, it is possible to use the nucleic acid molecules according to the invention as molecular markers or primers in plant breeding. Suitable means for detection are well known to a person skilled in the art, e.g. buffers and solutions for hybridization assays, e.g. the afore-mentioned solutions and buffers, further and means for Southern-, Western-, Northern- etc. -blots, as e.g.
30 described in Sambrook et al. are known. In one embodiment diagnostic composition contain PCR primers designed to specifically detect the presence or the expression level of the nucleic acid molecule to be reduced in the process of the invention, e.g. of the nucleic acid molecule of the invention, or to discriminate between different variants or alleles of the nucleic acid molecule of the invention or which activity is to be reduced
35 in the process of the invention.

[0235.4.1.1] In another embodiment, the present invention relates to a kit comprising the nucleic acid molecule, the vector, the host cell, the polypeptide, or the antisense, RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, cosuppression molecule, or ribozyme molecule, or the viral nucleic acid molecule, the antibody, plant cell, the plant

or plant tissue, the harvestable part, the propagation material and/or the compound and/or agonist identified according to the method of the invention.

The compounds of the kit of the present invention may be packaged in containers such as vials, optionally with/in buffers and/or solution. If appropriate, one or more of said components might be packaged in one and the same container. Additionally or alternatively, one or more of said components might be adsorbed to a solid support as, e.g. a nitrocellulose filter, a glass plate, a chip, or a nylon membrane or to the well of a micro titerplate. The kit can be used for any of the herein described methods and embodiments, e.g. for the production of the host cells, transgenic plants, pharmaceutical compositions, detection of homologous sequences, identification of antagonists or agonists, as food or feed or as a supplement thereof or as supplement for the treating of plants, etc.

Further, the kit can comprise instructions for the use of the kit for any of said embodiments.

In one embodiment said kit comprises further a nucleic acid molecule encoding one or more of the aforementioned protein, and/or an antibody, a vector, a host cell, an antisense nucleic acid, a plant cell or plant tissue or a plant. In another embodiment said kit comprises PCR primers to detect and discriminate the nucleic acid molecule to be reduced in the process of the invention, e.g. of the nucleic acid molecule of the invention.

[0235.5.1.1] In a further embodiment, the present invention relates to a method for the production of an agricultural composition providing the nucleic acid molecule for the use according to the process of the invention, the nucleic acid molecule of the invention, the vector of the invention, the antisense, RNAi, snRNA, dsRNA, siRNA, miRNA, ta-siRNA, cosuppression molecule, ribozyme, or antibody of the invention, the viral nucleic acid molecule of the invention, or the polypeptide of the invention or comprising the steps of the method according to the invention for the identification of said compound or agonist; and formulating the nucleic acid molecule, the vector or the polypeptide of the invention or the agonist, or compound identified according to the methods or processes of the present invention or with use of the subject matters of the present invention in a form applicable as plant agricultural composition.

[0235.6.1.1] In another embodiment, the present invention relates to a method for the production of the plant culture composition comprising the steps of the method of the present invention; and formulating the compound identified in a form acceptable as agricultural composition.

Under "acceptable as agricultural composition" is understood, that such a composition is in agreement with the laws regulating the content of fungicides, plant nutrients, herbicides, etc. Preferably such a composition is without any harm for the protected plants and the animals (humans included) fed therewith.

[0236.1.1.1] Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

- 5 It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes and variations may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples, which are not to be construed in any way as limiting. On the contrary, it is to be clearly understood that various other embodiments, modifications and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the claims.

[0237.1.1.1] **Example 1**

- 15 **Engineering stress-tolerant Arabidopsis plants by over-expressing genes coding for BSRP.**

[0238.1.1.1] **Example 1:** Cloning of the inventive sequences according to the SEQ ID NO: 38 or SEQ ID NO: 1660 respectively for the expression in plants

- Unless otherwise specified, standard methods as described in Sambrook et al., Molecular Cloning: A laboratory manual, Cold Spring Harbor 1989, Cold Spring Harbor Laboratory Press are used.

The inventive sequences were amplified by PCR as described in the protocol of the *Pfu* Ultra, *Pfu* Turbo or Herculase DNA polymerase (Stratagene).

- The composition for the protocol of the *Pfu* Ultra, *Pfu* Turbo or Herculase DNA polymerase was as follows: 1x PCR buffer (Stratagene), 0.2 mM of each dNTP, 100 ng genomic DNA of *Saccharomyces cerevisiae* (strain S288C; Research Genetics, Inc., now Invitrogen) or *Escherichia coli* (strain MG1655; *E.coli* Genetic Stock Center), 50 pmol forward primer, 50 pmol reverse primer, 2.5 u *Pfu* Ultra, *Pfu* Turbo or Herculase DNA polymerase.

The amplification cycles were as follows:

- 30 *Saccharomyces cerevisiae*: 1 cycle of 2-3 minutes at 94-95°C, followed by 25-36 cycles of in each case 1 minute at 95°C or 30 seconds at 94°C, 45 seconds at 50°C, 30 seconds at 50°C or 30 seconds at 55°C and 210-480 seconds at 72°C, followed by 1 cycle of 8 minutes at 72°C, then 4°C.

- 35 *Escherichia coli* : 1 cycle of 2-3 minutes at 94°C, followed by 25-30 cycles of in each case 30 seconds at 94°C, 30 seconds at 55-60°C and 5-10 minutes at 72°C, followed by 1 cycle of 10 minutes at 72°C, then 4°C.

The The following adapter sequences were added to *Saccharomyces cerevisiae* ORF specific primers (see table IV) for cloning purposes:

- i) forward primer: 5'-GGAATTCCAGCTGACCACC-3'
SEQ ID NO: 10
- 5 ii) reverse primer: 5'-GATCCCCGGGAATTGCCATG-3'
SEQ ID NO: 11

The following adapter sequences were added to *Escherichia coli* ORF specific primers for cloning purposes:

- 10 iii) forward primer: 5'-TTGCTCTTCC- 3'
SEQ ID NO: 12
- iiii) reverse primer: 5'-TTGCTCTTCG-3'
SEQ ID NO: 13

- 15 Therefore for amplification and cloning of *Saccharomyces cerevisiae* SEQ ID NO: 1660, a primer consisting of the adaptor sequence i) and the ORF specific sequence SEQ ID NO: 1662 and a second primer consisting of the adaptor sequence ii) and the ORF specific sequence SEQ ID NO: 1663 were used.

- 20 For amplification and cloning of *Escherichia coli* SEQ ID NO: 38 a primer consisting of the adaptor sequence iii) and the ORF specific sequence SEQ ID NO: 130 and a second primer consisting of the adaptor sequence iiiii) and the ORF specific sequence SEQ ID NO: 131 were used.

25 [0239.1.1.1] Construction of binary vectors for non-targeted expression of proteins

"Non-targeted" expression in this context means, that no additional targeting sequence was added to the ORF to be expressed.

- 30 For non-targeted constitutive expression in preferentially green tissues the following binary vectors were used for cloning: pMTX155 SEQ ID NO 1 and VC-MME220-1qcz SEQ ID NO 4.

For cloning the ORFs from yeast, the binary vector pMTX155 containing the enhanced 35S (Big35S) promoter SEQ ID NO: 8 (Comai et al., Plant Mol Biol 15, 373-383 (1990) was used.

For cloning the ORFs from *E.coli*, the binary vector VC-MME220-1qcz containing the super promoter SEQ ID NO: 9 (Ni et al., Plant Journal 7, 661 (1995), WO 95/14098) was used.

- 5 Other useful binary vectors are known to the skilled worker; an overview of binary vectors and their use can be found in Hellens, R., Mullineaux, P. and Klee H., ((2000) "A guide to Agrobacterium binary vectors", Trends in Plant Science, Vol. 5 No.10, 446–451]. Such vectors have to be equally equipped with appropriate promoters and targeting sequences.

- 10 **[0239.2.1.1]** Construction of binary vectors for targeting of expressed proteins to the plastids.

For constitutive expression the binary vectors used for cloning the targeting sequence were VC-MME354-1QCZ SEQ ID NO: 2 and VC-MME432-1qcz SEQ ID NO: 5.

- 15 Amplification of the plastidic targeting sequence of the gene FNR from *Spinacia oleracea* and construction of vector for plastid-targeted expression in preferential green tissues.

In order to amplify the targeting sequence of the FNR gene from *S. oleracea*, genomic DNA was extracted from leaves of 4 weeks old *S. oleracea* plants (DNeasy Plant Mini Kit, Qiagen, Hilden). The gDNA was used as the template for a PCR.

- 20 To enable cloning of the transit sequence into the vector VC-MME489-1QCZ SEQ ID NO: 7 an EcoRI restriction enzyme recognition sequence was added to both the forward and reverse primers, whereas for cloning into the vector VC-MME220-1qcz SEQ ID NO: 4 a PmeI restriction enzyme recognition sequence was added to the forward primer and a NcoI site was added to the reverse primer.

- 25 FNR5EcoResgen ATA gAA TTC gCA TAA ACT TAT CTT CAT AgT TgC C
SEQ ID NO: 14

FNR3EcoResgen ATA gAA TTC AgA ggC gAT CTg ggC CCT
SEQ ID NO: 15

- FNR5PmeColic ATA gTT TAA ACg CAT AAA CTT ATC TTC ATA gTT gCC
SEQ ID NO: 16
30 FNR3NcoColic ATA CCA Tgg AAg AgC AAg Agg CgA TCT ggg CCC T
SEQ ID NO: 17

The sequence SEQ ID NO: 18 amplified from genomic spinach DNA, comprised a 5'UTR (bp1-166), and the coding region (bp 166-273 and 351-419). The coding sequence is interrupted by an intronic sequence from bp 274 to-bp 350.

gcataaacttatctcatagttgccactccaatttgctccttgaatctcctccaccaatacataatccactcctccatcaccca
 5 cttcactactaaatcaaacttaactctgttttctctcctcttcatttcttattctccaatcatcgctactccgccatgaccaccg
ctgtcaccgccgctgtttcttcccctctacaaaaccacctctctccgccgaagctcctccgtcatttcccctgacaaaa
tcagctacaaaaagggtattcccaattcactgtgttttattaataattgttatttgatgatgagatgattaattgggtgctgc
aggttcctttgtactacaggaatgtatctgcaactgggaaaatgggacccatcagggcccagatcgccctct

The PCR fragment derived with the primers FNR5EcoResgen and FNR3EcoResgen
 10 was digested with EcoRI and ligated in the vector VC-MME489-1QCZ, that had also
 been digested with EcoRI. The correct orientation of the FNR targeting sequence was
 tested by sequencing. The vector generated in this ligation step was VC-MME354-
 1QCZ SEQ ID NO: 2.

The PCR fragment derived with the primers FNR5PmeColic and FNR3NcoColic was
 15 digested with PmeI and NcoI and ligated into the vector VC-MME220-1qcz that had
 been digested with SmaI and NcoI. The vector generated in this ligation step was VC-
 MME432-1qcz SEQ ID NO: 5.

[0240.1.1.1] For cloning ORFs from *S. cerevisiae* amplified as described above
 20 into a vector containing the *Resgen* adapter sequence (pMTX155) the vector DNA was
 treated with the restriction enzyme NcoI. For cloning of ORFs from *E. coli* into the
 vectors containing the *Colic* adapter sequence (VC-MME220-1qcz), the vector DNA
 was treated with the restriction enzymes PacI and NcoI following the standard protocol
 (MBI Fermentas). In all cases the reaction was stopped by inactivation at 70°C for 20
 25 minutes and purified over QIAquick or NucleoSpin Extract II columns following the
 standard protocol (Qiagen or Macherey-Nagel).

Then the PCR-product representing the amplified ORF with the respective adapter se-
 quences and the vector DNA were treated with T4 DNA polymerase according to the
 standard protocol (MBI Fermentas) to produce single stranded overhangs with the pa-
 30 rameters 1 unit T4 DNA polymerase at 37°C for 2-10 minutes for the vector and 1-2 u
 T4 DNA polymerase at 15-17°C for 10-60 minutes for the PCR product representing
 the ORF.

The reaction was stopped by addition of high-salt buffer and purified over QIAquick
 QIAquick or NucleoSpin Extract II columns following the standard protocol (Qiagen or
 35 Macherey-Nagel).

Approximately 30-60 ng of prepared vector and a defined amount of prepared amplificate were mixed and hybridized at 65°C for 15 minutes followed by 37°C 0,1 °C/1 seconds, followed by 37°C 10 minutes, followed by 0,1 °C/1 seconds, then 4-10 °C.

5 The ligated constructs were transformed in the same reaction vessel by addition of competent *E. coli* cells (strain DH5alpha) and incubation for 20 minutes at 1°C followed by a heat shock for 90 seconds at 42°C and cooling to 1-4°C. Then, complete medium (SOC) was added and the mixture was incubated for 45 minutes at 37°C. The entire mixture was subsequently plated onto an agar plate with 0.05 mg/ml kanamycin and incubated overnight at 37°C.

10 The outcome of the cloning step was verified by amplification with the aid of primers which bind upstream and downstream of the integration site, thus allowing the amplification of the insertion. The amplifications were carried out as described in the protocol of *Taq* DNA polymerase (Gibco-BRL).

15 The amplification cycles were as follows: 1 cycle of 1-5 minutes at 94°C, followed by 35 cycles of in each case 15-60 seconds at 94°C, 15-60 seconds at 50-66°C and 5-15 minutes at 72°C, followed by 1 cycle of 10 minutes at 72°C, then 4-16°C.

Several colonies were checked, but only one colony for which a PCR product of the expected size was detected was used in the following steps.

20 A portion of this positive colony was transferred into a reaction vessel filled with complete medium (LB) supplemented with kanamycin (50 µg/ml) and incubated overnight at 37°C.

The plasmid preparation was carried out as specified in the Qiaprep or NucleoSpin Multi-96 Plus standard protocol (Qiagen or Macherey-Nagel).

25 **[0241.1.1.1] Example 2:** Generation of transgenic plants which express
SEQ ID NO: 38 or SEQ ID NO: 1660 respectively

30 1-5 ng of the plasmid DNA isolated was transformed by electroporation or transformation into competent cells of *Agrobacterium tumefaciens*, of strain GV 3101 pMP90 (Koncz and Schell, Mol. Gen. Gent. 204, 383, 1986). Thereafter, complete medium (YEP) was added and the mixture was transferred into a fresh reaction vessel for 3 hours at 28°C. Thereafter, all of the reaction mixture was plated onto YEP agar plates supplemented with the respective antibiotics, e.g. rifampicine (0.1 mg/ml), gentamycine (0.025 mg/ml and kanamycine (0.05 mg/ml) and incubated for 48 hours at 28°C.

The agrobacteria that contains the plasmid construct were then used for the transformation of plants.

A colony was picked from the agar plate with the aid of a pipette tip and taken up in 3 ml of liquid TB medium, which also contained suitable antibiotics as described above. The preculture was grown for 48 hours at 28°C and 120 rpm.

- 5 400 ml of LB medium containing the same antibiotics as above were used for the main culture. The preculture was transferred into the main culture. It was grown for 18 hours at 28°C and 120 rpm. After centrifugation at 4 000 rpm, the pellet was resuspended in infiltration medium (MS medium, 10% sucrose).

- 10 In order to grow the plants for the transformation, dishes (Piki Saat 80, green, provided with a screen bottom, 30 x 20 x 4.5 cm, from Wiesauplast, Kunststofftechnik, Germany) were half-filled with a GS 90 substrate (standard soil, Werkverband E.V., Germany). The dishes were watered overnight with 0.05% Proplant solution (Chimac-Apriphar, Belgium). *A. thaliana* C24 seeds (Nottingham Arabidopsis Stock Centre, UK ; NASC Stock N906) were scattered over the dish, approximately 1000 seeds per dish. The dishes were covered with a hood and placed in the stratification facility (8 h, 110
15 $\mu\text{mol}/\text{m}^2\text{s}$, 22°C; 16 h, dark, 6°C). After 5 days, the dishes were placed into the short-day controlled environment chamber (8 h, 130 $\mu\text{mol}/\text{m}^2\text{s}^1$, 22°C; 16 h, dark, 20°C), where they remained for approximately 10 days until the first true leaves had formed.

- 20 The seedlings were transferred into pots containing the same substrate (Teku pots, 7 cm, LC series, manufactured by Pöppelmann GmbH & Co, Germany). Five plants were pricked out into each pot. The pots were then returned into the short-day controlled environment chamber for the plant to continue growing.

After 10 days, the plants were transferred into the greenhouse cabinet (supplementary illumination, 16 h, 340 $\mu\text{E}/\text{m}^2\text{s}$, 22°C; 8 h, dark, 20°C), where they were allowed to grow for further 17 days.

- 25 For the transformation, 6-week-old Arabidopsis plants, which had just started flowering were immersed for 10 seconds into the above-described agrobacterial suspension which had previously been treated with 10 μl Silwett L77 (Crompton S.A., Osi Specialties, Switzerland). The respective method is described in Clough and Bent, 1998 (Clough, JC and Bent, AF. 1998 Floral dip: a simplified method for *Agrobacterium*-
30 mediated transformation of *Arabidopsis thaliana*, Plant J. 16:735-743).

The plants were subsequently placed for 18 hours into a humid chamber. Thereafter, the pots were returned to the greenhouse for the plants to continue growing. The plants remained in the greenhouse for another 10 weeks until the seeds were ready for harvesting.

- 35 Depending on the resistance marker used for the selection of the transformed plants the harvested seeds were planted in the greenhouse and subjected to a spray selection or else first sterilized and then grown on agar plates supplemented with the re-

spective selection agent. Since the vector contained the bar gene as the resistance marker, plantlets were sprayed four times at an interval of 2 to 3 days with 0.02 % BASTA® and transformed plants were allowed to set seeds. The plants remained in the greenhouse for another 10 weeks until the seeds were ready for harvesting. The seeds of the transgenic *A. thaliana* plants were stored in the freezer (at -20°C).

[0242.1.1.1] **Example 3: Fungal Resistance Screen**

[0243.1.1.1] **Plant growth and experiment design**

In a standard experiment nutrient rich soil (GS90, Tantau, Wansdorf, Germany) were used. Pots were filled with soil and placed into trays. Water was added to the trays to let the soil take up appropriate amount of water for the sowing procedure. The seeds for transgenic *Arabidopsis thaliana* C24 plants were sown in pots (10 cm diameter). Then the filled tray was covered with a transparent lid without holes and transferred into the shelf system of the growth chamber. Lid and pots were sprayed daily. Stratification was established for a period of 4 days in the dark at 4°C for 8 h, in the light (220 $\mu\text{mol}/\text{m}^2\text{s}$) at 20°C for 16 h, relative humidity with 68%. After 4 days the parameter were changed to 12 h light and 12 h darkness and covers were removed. BASTA selection was done at day 5, 7, 10 and 12 after sowing by spraying pots with plantlets from the top. Therefore, a 0.02% (v/v) solution of BASTA concentrate (183 g/l glufosinate-ammonium) in tap water was sprayed. The location of the trays inside the chambers was changed on working days after sowing. Watering were done as necessary after covers were removed from the trays. Plants were piqued 14 days after sowing in another pot with GS90, 5 plants together in 1 pot and 3 pots per line. 4 lines and 1 wild type (in the middle) were placed together in 1 tray. The tray was covered with the lid and sprayed for 3 days. Positive (sensitive = pad3-1-wt-F1-(1)) and negative (resistant = Col-0-Lehle-F1-(1)) controls were in an extra tray. 28 days after sowing phenotypic information was valued in case of plants differ from the wild type control. 31 days after sowing tap water were added in the tray in a height of 1cm. A day later plants were sprayed 3 times with the fungal *Alternaria brassicicola* or *Alternaria alternata* (1.5×10^6 spores / ml) and covered again. Lid were sprayed 2 times a day with water for 2 days. 3 and 4 days after inoculation the evaluation were done.

[0244.1.1.1] **Cultivation of the fungus and inoculation**

The phytopathogenic fungi *Alternaria brassicicola* and *Alternaria alternata* were maintained on 2% Agar containing 2% malt-extract. The plates were incubated at 24° C with a 12h light/dark rhythm. To inoculate the plants, spore suspensions were prepared. Twelve-day-old fungal culture plates were rinsed with 2% malt-extract solution. The crude spore-mycelium suspension were filtered through 1 layer of gauze. The spore densities were determined by counting in a Thoma-chamber. A freshly harvested spore

suspension with a density of $1,5 \times 10^6$ Spores/ml was used to inoculate well watered plants.

[0245.1.1.1] Scoring of the plants symptoms

- 5 The disease symptoms were scored in comparison to untransformed wild type control plants. The range of scoring were between 1 and 5. Leafs with symptoms of chlorotic areas and mazaration of the tissue were counted of all three pots. The phenotype (big-ness and habitus) of the plants had an influence to the final scoring.

Table 2:

- 10 List of ORF (Column 1) from yeast (*S. cerevisiae*) or *E. coli* (Column 2) which resulted in enhanced fungal resistance as shown in column 4 when expressed as indicated in column 3 (without artificial targeting of with plastidic targeting) in comparison to non-transformed wild type plants.

Orf	Organism	Artificial Target	Enhanced Resistance against Alternaria species
b0124	<i>E. coli</i>	non	Alternata and brassicicola
b0730	<i>E. coli</i>	non	Alternata and brassicicola
b1528	<i>E. coli</i>	non	Alternata and brassicicola
b2148	<i>E. coli</i>	non	Alternata and brassicicola
b2573	<i>E. coli</i>	non	Alternata and brassicicola
b2600	<i>E. coli</i>	plastidic	Alternata and brassicicola
b2699	<i>E. coli</i>	non	Alternata and brassicicola
b2764	<i>E. coli</i>	non	Alternata or brassicicola
b3262	<i>E. coli</i>	non	Alternata and brassicicola
b3363	<i>E. coli</i>	non	Alternata or brassicicola
b3644	<i>E. coli</i>	non	Alternata or brassicicola
YCR087W	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YGR150C	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YIL172C	<i>S. cerevisiae</i>	non	Alternata or brassicicola
YLR168C	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YLR407W	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YNL241C	<i>S. cerevisiae</i>	plastidic	Alternata and brassicicola
YOR260W	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YOR299W	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YPL079W	<i>S. cerevisiae</i>	non	Alternata and brassicicola
YPL151C	<i>S. cerevisiae</i>	non	Alternata and brassicicola

Unless indicated otherwise, chemicals and reagents in the Examples were obtained from Sigma Chemical Company (St. Louis, MO), restriction endonucleases were from New England Biolabs (Beverly, MA) or Roche (Indianapolis, IN), oligonucleotides were synthesized by MWG Biotech Inc. (High Point, NC), and other modifying enzymes or kits regarding biochemicals and molecular biological assays were from Clontech (Palo Alto, CA), Pharmacia Biotech (Piscataway, NJ), Promega Corporation (Madison, WI), or Stratagene (La Jolla, CA). Materials for cell culture media were obtained from Gibco/BRL (Gaithersburg, MD) or DIFCO (Detroit, MI). The cloning steps carried out for the purposes of the present invention, such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of *E. coli* cells, growing bacteria, multiplying phages and sequence analysis of recombinant DNA, are carried out as described by Sambrook (1989). The sequencing of recombinant DNA molecules is carried out using ABI laser fluorescence DNA sequencer following the method of Sanger (Sanger 1977).

[0247.1.1.1] Example 4

Engineering alfalfa plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

A regenerating clone of alfalfa (*Medicago sativa*) is transformed using the method of (McKersie et al., 1999 Plant Physiol 119: 839–847). Regeneration and transformation of alfalfa is genotype dependent and therefore a regenerating plant is required. Methods to obtain regenerating plants have been described. For example, these can be selected from the cultivar Rangelander (Agriculture Canada) or any other commercial alfalfa variety as described by Brown DCW and A Atanassov (1985. Plant Cell Tissue Organ Culture 4: 111-112). Alternatively, the RA3 variety (University of Wisconsin) is selected for use in tissue culture (Walker et al., 1978 Am J Bot 65:654-659).

Petiole explants are cocultivated with an overnight culture of *Agrobacterium tumefaciens* C58C1 pMP90 (McKersie et al., 1999 Plant Physiol 119: 839–847) or LBA4404 containing a binary vector. Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols. Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the vector pBIN19 described by Bevan (Nucleic Acid Research. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic

DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and 6225105). Similarly, various promoters can be used to regulate the trait gene that provides constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) is used to provide constitutive expression of the trait gene.

The explants are cocultivated for 3 d in the dark on SH induction medium containing 288 mg/ L Pro, 53 mg/ L thioproline, 4.35 g/ L K₂SO₄, and 100 µm acetosyringinone. The explants are washed in half-strength Murashige-Skoog medium (Murashige and Skoog, 1962) and plated on the same SH induction medium without acetosyringinone but with a suitable selection agent and suitable antibiotic to inhibit Agrobacterium growth. After several weeks, somatic embryos are transferred to BOi2Y development medium containing no growth regulators, no antibiotics, and 50 g/ L sucrose. Somatic embryos are subsequently germinated on half-strength Murashige-Skoog medium. Rooted seedlings are transplanted into pots and grown in a greenhouse.

The T₀ transgenic plants are propagated by node cuttings and rooted in Turface growth medium. The plants are defoliated and grown to a height of about 10 cm (approximately 2 weeks after defoliation). The plants are then subjected to plant disease, preferably pathogenic fungi.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

Example 5

Engineering ryegrass plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

Seeds of several different ryegrass varieties may be used as explant sources for transformation, including the commercial variety Gunne available from Svalof Weibull seed company or the variety Affinity. Seeds are surface-sterilized sequentially with 1% Tween-20 for 1 minute, 100 % bleach for 60 minutes, 3 rinses with 5 minutes each with de-ionized and distilled H₂O, and then germinated for 3-4 days on moist, sterile filter paper in the dark. Seedlings are further sterilized for 1 minute with 1% Tween-20, 5 minutes with 75% bleach, and rinsed 3 times with ddH₂O, 5 min each.

Surface-sterilized seeds are placed on the callus induction medium containing Murashige and Skoog basal salts and vitamins, 20 g/l sucrose, 150 mg/l asparagine, 500

mg/l casein hydrolysate, 3 g/l Phytigel, 10 mg/l BAP, and 5 mg/l dicamba. Plates are incubated in the dark at 25°C for 4 weeks for seed germination and embryogenic callus induction.

- After 4 weeks on the callus induction medium, the shoots and roots of the seedlings are trimmed away, the callus is transferred to fresh media, maintained in culture for another 4 weeks, and then transferred to MSO medium in light for 2 weeks. Several pieces of callus (11-17 weeks old) are either strained through a 10 mesh sieve and put onto callus induction medium, or cultured in 100 ml of liquid ryegrass callus induction media (same medium as for callus induction with agar) in a 250 ml flask. The flask is wrapped in foil and shaken at 175 rpm in the dark at 23 °C for 1 week. Sieving the liquid culture with a 40-mesh sieve collected the cells. The fraction collected on the sieve is plated and cultured on solid ryegrass callus induction medium for 1 week in the dark at 25°C. The callus is then transferred to and cultured on MS medium containing 1% sucrose for 2 weeks.
- Transformation can be accomplished with either *Agrobacterium* or with particle bombardment methods. An expression vector is created containing a constitutive plant promoter and the cDNA of the gene in a pUC vector. The plasmid DNA is prepared from *E. coli* cells using Qiagen kit according to manufacturer's instruction. Approximately 2 g of embryogenic callus is spread in the center of a sterile filter paper in a Petri dish. An aliquot of liquid MSO with 10 g/l sucrose is added to the filter paper. Gold particles (1.0 µm in size) are coated with plasmid DNA according to method of Sanford et al., 1993 and delivered to the embryogenic callus with the following parameters: 500 µg particles and 2 µg DNA per shot, 1300 psi and a target distance of 8.5 cm from stopping plate to plate of callus and 1 shot per plate of callus.
- After the bombardment, calli are transferred back to the fresh callus development medium and maintained in the dark at room temperature for a 1-week period. The callus is then transferred to growth conditions in the light at 25 °C to initiate embryo differentiation with the appropriate selection agent, e.g. 250 nM Arsenal, 5 mg/l PPT or 50 mg/L kanamycin. Shoots resistant to the selection agent appear and once rooted are transferred to soil.
- Samples of the primary transgenic plants (T0) are analyzed by PCR to confirm the presence of T-DNA. These results are confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.
- Transgenic T0 ryegrass plants are propagated vegetatively by excising tillers. The transplanted tillers are maintained in the greenhouse for 2 months until well established. The shoots are defoliated and allowed to grow for 2 weeks.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

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Example 6

Engineering rice plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

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Rice transformation

The *Agrobacterium* containing the expression vector of the invention is used to transform *Oryza sativa* plants. Mature dry seeds of the rice japonica cultivar Nipponbare are dehusked. Sterilization is carried out by incubating for one minute in 70% ethanol, followed by 30 minutes in 0.2% HgCl_2 , followed by a 6 times 15 minutes wash with sterile distilled water. The sterile seeds are then germinated on a medium containing 2,4-D (callus induction medium). After incubation in the dark for four weeks, embryogenic, scutellum-derived calli are excised and propagated on the same medium. After two weeks, the calli are multiplied or propagated by subculture on the same medium for another 2 weeks. Embryogenic callus pieces are sub-cultured on fresh medium 3 days before co-cultivation (to boost cell division activity).

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Agrobacterium strain LBA4404 containing the expression vector of the invention is used for co-cultivation. *Agrobacterium* is inoculated on AB medium with the appropriate antibiotics and cultured for 3 days at 28°C. The bacteria are then collected and suspended in liquid co-cultivation medium to a density (OD_{600}) of about 1. The suspension is then transferred to a Petri dish and the calli immersed in the suspension for 15 minutes. The callus tissues are then blotted dry on a filter paper and transferred to solidified, co-cultivation medium and incubated for 3 days in the dark at 25°C. Co-cultivated calli are grown on 2,4-D-containing medium for 4 weeks in the dark at 28°C in the presence of a selection agent. During this period, rapidly growing resistant callus islands developed. After transfer of this material to a regeneration medium and incubation in the light, the embryogenic potential is released and shoots developed in the next four to five weeks. Shoots are excised from the calli and incubated for 2 to 3 weeks on an auxin-containing medium from which they are transferred to soil. Hardened shoots are grown under high humidity and short days in a greenhouse.

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Approximately 35 independent T0 rice transformants are generated for one construct. The primary transformants are transferred from a tissue culture chamber to a greenhouse. After a quantitative PCR analysis to verify copy number of the T-DNA insert,

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only single copy transgenic plants that exhibit tolerance to the selection agent are kept for harvest of T1 seed. Seeds are then harvested three to five months after transplanting. The method yielded single locus transformants at a rate of over 50 % (Aldemita and Hodges 1996, Chan et al. 1993, Hiei et al. 1994).

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Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

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Example 7

Engineering soybean plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

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Soybean is transformed according to the following modification of the method described in the Texas A&M patent US 5,164,310. Several commercial soybean varieties are amenable to transformation by this method. The cultivar Jack (available from the Illinois Seed Foundation) is a commonly used for transformation. Seeds are sterilized by immersion in 70% (v/v) ethanol for 6 min and in 25 % commercial bleach (NaOCl) supplemented with 0.1% (v/v) Tween for 20 min, followed by rinsing 4 times with sterile double distilled water. Seven-day seedlings are propagated by removing the radicle, hypocotyl and one cotyledon from each seedling. Then, the epicotyl with one cotyledon is transferred to fresh germination media in petri dishes and incubated at 25 °C under a 16-hr photoperiod (approx. 100 μ E-m-2s-1) for three weeks. Axillary nodes (approx. 4 mm in length) were cut from 3 – 4 week-old plants. Axillary nodes are excised and incubated in *Agrobacterium* LBA4404 culture.

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Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols. Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the vector pBIN19 described by Bevan (Nucleic Acid Research. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and 6225105). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene tran-

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scription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) can be used to provide constitutive expression of the trait gene.

After the co-cultivation treatment, the explants are washed and transferred to selection media supplemented with 500 mg/L timentin. Shoots are excised and placed on a shoot elongation medium. Shoots longer than 1 cm are placed on rooting medium for two to four weeks prior to transplanting to soil.

The primary transgenic plants (T0) are analyzed by PCR to confirm the presence of T-DNA. These results are confirmed by Southern hybridization in which DNA is electrophoresed on a 1 % agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

Example 8

Engineering Rapeseed/Canola plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

Cotyledonary petioles and hypocotyls of 5-6 day-old young seedlings are used as explants for tissue culture and transformed according to Babic et al.(1998, Plant Cell Rep 17: 183-188). The commercial cultivar Westar (Agriculture Canada) is the standard variety used for transformation, but other varieties can be used.

Agrobacterium tumefaciens LBA4404 containing a binary vector can be used for canola transformation. Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols. Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the vector pBIN19 described by Bevan (Nucleic Acid Research. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and 6225105). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene

transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) can be used to provide constitutive expression of the trait gene.

Canola seeds are surface-sterilized in 70% ethanol for 2 min., and then in 30% Clorox with a drop of Tween-20 for 10 min, followed by three rinses with sterilized distilled water. Seeds are then germinated in vitro 5 days on half strength MS medium without hormones, 1% sucrose, 0.7% Phytagar at 23°C, 16 hr. light. The cotyledon petiole explants with the cotyledon attached are excised from the in vitro seedlings, and inoculated with *Agrobacterium* by dipping the cut end of the petiole explant into the bacterial suspension. The explants are then cultured for 2 days on MSBAP-3 medium containing 3 mg/l BAP, 3 % sucrose, 0.7 % Phytagar at 23 °C, 16 hr light. After two days of co-cultivation with *Agrobacterium*, the petiole explants are transferred to MSBAP-3 medium containing 3 mg/l BAP, cefotaxime, carbenicillin, or timentin (300 mg/l) for 7 days, and then cultured on MSBAP-3 medium with cefotaxime, carbenicillin, or timentin and selection agent until shoot regeneration. When the shoots were 5 – 10 mm in length, they are cut and transferred to shoot elongation medium (MSBAP-0.5, containing 0.5 mg/l BAP). Shoots of about 2 cm in length are transferred to the rooting medium (MS0) for root induction.

Samples of the primary transgenic plants (T0) are analyzed by PCR to confirm the presence of T-DNA. These results are confirmed by Southern hybridization in which DNA is electrophoresed on a 1 % agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

Example 9

Engineering corn plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example

Transformation of maize (*Zea Mays* L.) is performed with a modification of the method described by Ishida et al. (1996. *Nature Biotech* 14:745-50). Transformation is genotype-dependent in corn and only specific genotypes are amenable to transformation and regeneration. The inbred line A188 (University of Minnesota) or hybrids with A188 as a parent are good sources of donor material for transformation (Fromm et al. 1990 *Biotech* 8:833-839), but other genotypes can be used successfully as well. Ears are harvested from corn plants at approximately 11 days after pollination (DAP) when the length of immature embryos is about 1 to 1.2 mm. Immature embryos are co-cultivated

with *Agrobacterium tumefaciens* that carry "super binary" vectors and transgenic plants are recovered through organogenesis. The super binary vector system of Japan Tobacco is described in WO patents WO94/00977 and WO95/06722. Vectors were constructed as described. Various selection marker genes can be used including the maize gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patent 6025541). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

- Excised embryos are grown on callus induction medium, then maize regeneration medium, containing imidazolinone as a selection agent. The Petri plates are incubated in the light at 25 °C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to maize rooting medium and incubated at 25 °C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the imidazolinone herbicides and which are PCR positive for the transgenes.

The T1 transgenic plants are then evaluated for their improved stress tolerance.

- Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants.

Example 10

- Engineering wheat plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing stress related genes from *Saccharomyces cerevisiae* or *E. coli* or from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* for example**

- Transformation of wheat is performed with the method described by Ishida et al. (1996 Nature Biotech. 14745-50). The cultivar Bobwhite (available from CYMMIT, Mexico) is commonly used in transformation. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. The super binary vector system of Japan Tobacco is described in WO patents WO94/00977 and WO95/06722. Vectors were constructed as described. Various selection marker genes can be used including the maize gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patent 6025541). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

After incubation with *Agrobacterium*, the embryos are grown on callus induction medium, then regeneration medium, containing imidazolinone as a selection agent. The Petri plates are incubated in the light at 25 °C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to rooting medium and incubated
5 at 25 °C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the imidazolinone herbicides and which are PCR positive for the transgenes.

The T1 transgenic plants are then evaluated for their improved stress tolerance according to the method described in the previous example 3. The T1 generation of single
10 locus insertions of the T-DNA will segregate for the transgene in a 3:1 ratio. Those progeny containing one or two copies of the transgene are tolerant of the imidazolinone herbicide, and exhibit greater tolerance of biotic stress or drought stress than those progeny lacking the transgenes. Tolerant plants have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter
15 production than susceptible plants. Homozygous T2 plants exhibited similar phenotypes.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait
20 including seed yield, photosynthesis and dry matter production than susceptible plants

Example 11

Identification of Identical and Heterologous Genes

Gene sequences can be used to identify identical or heterologous genes from cDNA or
25 genomic libraries. Identical genes (e. g. full-length cDNA clones) can be isolated via nucleic acid hybridization using for example cDNA libraries. Depending on the abundance of the gene of interest, 100,000 up to 1,000,000 recombinant bacteriophages are plated and transferred to nylon membranes. After denaturation with alkali, DNA is immobilized on the membrane by e. g. UV cross linking. Hybridization is carried out at
30 high stringency conditions. In aqueous solution, hybridization and washing is performed at an ionic strength of 1 M NaCl and a temperature of 68°C. Hybridization probes are generated by e.g. radioactive (³²P) nick transcription labeling (High Prime, Roche, Mannheim, Germany). Signals are detected by autoradiography.

Partially identical or heterologous genes that are related but not identical can be identified in a manner analogous to the above-described procedure using low stringency
35 hybridization and washing conditions. For aqueous hybridization, the ionic strength is normally kept at 1 M NaCl while the temperature is progressively lowered from 68 to 42°C.

Isolation of gene sequences with homology (or sequence identity/similarity) only in a distinct domain of (for example 10-20 amino acids) can be carried out by using synthetic radio labeled oligonucleotide probes. Radiolabeled oligonucleotides are prepared by phosphorylation of the 5-prime end of two complementary oligonucleotides with T4 polynucleotide kinase. The complementary oligonucleotides are annealed and ligated to form concatemers. The double stranded concatemers are then radiolabeled by, for example, nick transcription. Hybridization is normally performed at low stringency conditions using high oligonucleotide concentrations.

Oligonucleotide hybridization solution:

- 10 6 x SSC
- 0.01 M sodium phosphate
- 1 mM EDTA (pH 8)
- 0.5 % SDS
- 100 µg/ml denatured salmon sperm DNA
- 15 0.1 % nonfat dried milk

During hybridization, temperature is lowered stepwise to 5-10°C below the estimated oligonucleotide T_m or down to room temperature followed by washing steps and autoradiography. Washing is performed with low stringency such as 3 washing steps using 4 x SSC. Further details are described by Sambrook, J. et al., 1989, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al., 1994, "Current Protocols in Molecular Biology," John Wiley & Sons.

Example 12

25 **Identification of homologous or orthologous Genes by Screening Expression Libraries with Antibodies**

c-DNA clones can be used to produce recombinant polypeptide for example in *E. coli* (e.g. Qiagen QIAexpress pQE system). Recombinant polypeptides are then normally affinity purified via Ni-NTA affinity chromatography (Qiagen). Recombinant polypeptides are then used to produce specific antibodies for example by using standard techniques for rabbit immunization. Antibodies are affinity purified using a Ni-NTA column saturated with the recombinant antigen as described by Gu et al., 1994, BioTechniques 17:257-262. The antibody can then be used to screen expression cDNA libraries to identify orthologous or heterologous genes via an immunological screening (Sambrook, J. et al., 1989, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al., 1994, "Current Protocols in Molecular Biology", John Wiley & Sons).

Example 13

In vivo Mutagenesis

In vivo mutagenesis of microorganisms can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (e.g. *Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae*) which are impaired in their capabilities to maintain the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (e.g., mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D., 1996, DNA repair mechanisms, in: *Escherichia coli* and *Salmonella*, p. 2277-2294, ASM: Washington.) Such strains are well known to those skilled in the art. The use of such strains is illustrated, for example, in Greener, A. and Callahan, M., 1994, Strategies 7: 32-34. Transfer of mutated DNA molecules into plants is preferably done after selection and testing in microorganisms. Transgenic plants are generated according to various examples within the exemplification of this document.

Example 14

Engineering Arabidopsis plants with increased resistance to biotic stress, preferably pathogenic fungi by over-expressing biotic stress related protein encoding genes for example from *Brassica napus*, *Glycine max*, *Zea mays* or *Oryza sativa* using stress-inducible and tissue-specific promoters.

Transgenic Arabidopsis plants over-expressing stress related protein encoding genes from *Brassica napus*, *Glycine max*, *Zea mays* and *Oryza sativa* for example are created as described above to express the stress related protein encoding transgenes under the control of either a tissue-specific or stress-inducible promoter.

T2 generation plants are produced and treated with stress.

Tolerance of plant disease, preferably pathogenic fungi are measured using methods as described in example 3 or 7. Plants that have tolerance to plant disease, preferably pathogenic fungi have higher survival rates and preferably a further yield related trait including seed yield, photosynthesis and dry matter production than susceptible plants

Figures:

Fig. 1 Vector VC-MME220-1qcz (SEQ ID NO: 4) used for cloning gene of interest for non-targeted expression.

Fig. 2 Vector VC-MME354-1QCZ (SEQ ID NO: 2) used for cloning gene of interest for plastidic targeted expression.

Fig. 3 Vector VC-MME432-1qcz (SEQ ID NO: 5) used for cloning gene of interest for plastidic targeted expression.

Fig. 4 Vector VC-MME489-1QCZ (SEQ ID NO: 7) used for cloning gene of interest for non-targeted expression and cloning of a targeting sequence.

Fig. 5 Vector pMTX155 (SEQ ID NO: 1) used for used for cloning gene of interest for non-targeted expression.

Table IA: Nucleic acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
1	1	FR_OEX	B0124	E. coli	38	non-targeted	40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128
1	2	FR_OEX	B0730	E. coli	146	non-targeted	148, 150, 152, 154, 156
1	3	FR_OEX	B1528	E. coli	165	non-targeted	167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231
1	4	FR_OEX	B2148	E. coli	242	non-targeted	244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302
1	5	FR_OEX	B2573	E. coli	312	non-targeted	314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460
1	6	FR_OEX	B2600	E. coli	468	plastidic	470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522
1	7	FR_OEX	B2699	E. coli	534	non-targeted	536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 898, 900, 902, 904, 906, 908, 910, 912, 914, 916, 918, 920, 922, 924, 926, 928, 930, 932, 934, 936, 938, 940, 942, 944, 946, 948, 950, 952, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
							588, 590, 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, 640, 642, 644, 646, 648, 650, 652, 654, 656, 658, 660, 662, 664, 666, 668, 670, 672, 674, 676, 678, 680, 682, 684, 686, 688, 690, 692, 694, 696, 698, 700, 702, 704, 706, 708, 710, 712, 714, 716, 718, 720, 722, 724, 726, 728, 730, 732, 734, 736, 738, 740, 742, 744, 746, 748, 750, 752, 754, 756, 758, 760, 762, 764, 766, 768, 770, 772, 774, 776, 778, 780, 782, 784, 786, 788, 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828, 830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 898, 900, 902, 904, 906, 908, 910, 912, 914, 916, 918, 920, 922, 924, 926, 928, 930, 932, 934, 936
1	8	FR_OEX	B2764	E. coli	970	non-targeted	972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1068, 1070, 1072, 1074, 1076, 1078, 1080, 1082, 1084, 1086, 1088, 1090, 1092, 1094, 1096, 1098, 1100, 1102, 1104, 1106, 1108, 1110, 1112, 1114, 1116, 1118
1	9	FR_OEX	B3262	E. coli	1143	non-targeted	1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
1	10	FR_OEX	B3363	E. coli	1174	non-targeted	1176, 1178, 1180, 1182, 1184, 1186, 1188, 1190, 1192, 1194, 1196, 1198, 1200, 1202, 1204, 1206, 1208, 1210, 1212, 1214, 1216, 1218, 1220, 1222, 1224, 1226, 1228, 1230, 1232, 1234, 1236, 1238, 1240, 1242, 1244, 1246, 1248, 1250, 1252, 1254, 1256, 1258, 1260, 1262, 1264, 1266, 1268, 1270, 1272, 1274, 1276, 1278, 1280, 1282, 1284, 1286, 1288, 1290, 1292, 1294, 1296, 1298, 1300, 1302, 1304, 1306, 1308, 1310, 1312, 1314, 1316, 1318, 1320, 1322, 1324, 1326, 1328, 1330, 1332, 1334, 1336, 1338, 1340, 1342, 1344, 1346, 1348, 1350, 1352, 1354, 1356, 1358, 1360, 1362, 1364, 1366, 1368, 1370, 1372, 1374, 1376, 1378, 1380, 1382, 1384, 1386, 1388, 1390, 1392, 1394, 1396, 1398, 1400, 1402, 1404, 1406, 1408, 1410, 1412, 1414, 1416, 1418, 1420, 1422, 1424, 1426, 1428, 1430, 1432, 1434, 1436, 1438, 1440, 1442, 1444, 1446, 1448, 1450, 1452, 1454, 1456, 1458, 1460, 1462, 1464, 1466, 1468, 1470, 1472, 1474, 1476, 1478, 1480, 1482, 1484, 1486
	11	FR_OEX	B3644	E. coli	1521	non-targeted	1523, 1525, 1527, 1529, 1531, 1533, 1535, 1537, 1539, 1541, 1543, 1545, 1547, 1549, 1551, 1553, 1555, 1557, 1559, 1561, 1563, 1565, 1567, 1569, 1571, 1573, 1575, 1577, 1579, 1581, 1583, 1585, 1587, 1589, 1591, 1593, 1595, 1597, 1599, 1601, 1603, 1605, 1607, 1609, 1611, 1613, 1615, 1617, 1619, 1621, 1623, 1625, 1627, 1629, 1631, 1633, 1635, 1637, 1639, 1641, 1643, 1645, 1647, 1649, 1651

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
1	12	FR_OEX	YCR087W	S. cerevis iae	1660	non-targeted	-
1	13	FR_OEX	YGR150C	S. cerevis iae	1664	non-targeted	1666, 1668, 1670, 1672
1	14	FR_OEX	YIL172C	S. cerevis iae	1691	non-targeted	1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737, 1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797, 1799, 1801, 1803, 1805, 1807, 1809, 1811, 1813, 1815, 1817, 1819, 1821, 1823, 1825, 1827, 1829, 1831, 1833, 1835, 1837, 1839, 1841, 1843, 1845, 1847, 1849
1	15	FR_OEX	YLR168C	S. cerevis iae	1859	non-targeted	1861, 1863, 1865, 1867, 1869, 1871, 1873, 1875, 1877, 1879, 1881, 1883, 1885, 1887, 1889, 1891, 1893
1	16	FR_OEX	YLR407W	S. cerevis iae	1904	non-targeted	1906, 1908, 1910
1	17	FR_OEX	YNL241C	S. cerevis iae	1920	plastidic	1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1972, 1974, 1976, 1978, 1980,

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
							1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, 2082, 2084, 2086, 2088, 2090, 2092, 2094, 2096, 2098, 2100, 2102, 2104, 2106, 2108, 2110, 2112, 2114, 2116, 2118, 2120, 2122, 2124, 2126, 2128, 2130, 2132, 2134, 2136, 2138, 2140, 2142, 2144, 2146, 2148, 2150, 2152, 2154, 2156, 2158, 2160, 2162, 2164, 2166, 2168, 2170, 2172, 2174, 2176, 2178, 2180, 2182, 2184, 2186, 2188, 2190, 2192, 2194, 2196, 2198, 2200, 2202, 2204, 2206, 2208, 2210, 2212, 2214, 2216, 2218, 2220, 2222, 2224, 2226, 2228, 2230, 2232, 2234, 2236, 2238, 2240, 2242, 2244, 2246, 2248, 2250, 2252, 2254, 2256, 2258, 2260, 2262, 2264, 2266, 2268, 2270, 2272, 2274, 2276, 2278, 2280, 2282, 2284, 2286, 2288, 2290, 2292, 2294, 2296, 2298, 2300, 2302, 2304, 2306, 2308, 2310, 2312, 2314, 2316, 2318, 2320, 2322, 2324, 2326, 2328, 2330, 2332, 2334, 2336, 2338, 2340, 2342, 2344, 2346, 2348, 2350, 2352, 2354, 2356, 2358, 2360, 2362, 2364, 2366, 2368, 2370, 2372, 2374, 2376, 2378, 2380, 2382, 2384, 2386, 2388, 2390, 2392, 2394, 2396, 2398, 2400, 2402, 2404, 2406, 2408
1	18	FR_OEX	YOR260W	S.	2455	non-targeted	2457, 2459, 2461, 2463, 2465

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
				cerevis iae			
1	19	FR_OEX	YOR299W	S. cerevis iae	2477	non-targeted	2479, 2481, 2483, 2485, 2487, 2489, 2491
1	20	FR_OEX	YPL079W	S. cerevis iae	2507	non-targeted	2509, 2511, 2513, 2515, 2517, 2519, 2521, 2523, 2525, 2527, 2529, 2531, 2533, 2535, 2537, 2539, 2541, 2543, 2545, 2547, 2549, 2551, 2553, 2555, 2557, 2559, 2561, 2563, 2565, 2567, 2569, 2571, 2573, 2575, 2577, 2579, 2581, 2583, 2585, 2587, 2589, 2591, 2593, 2595, 2597, 2599, 2601, 2603, 2605, 2607, 2609, 2611, 2613, 2615, 2617, 2619, 2621, 2623, 2625, 2627, 2629, 2631, 2633, 2635
1	21	FR_OEX	YPL151C	S. cerevis iae	2683	non-targeted	2685, 2687, 2689, 2691, 2693, 2695, 2697, 2699, 2701, 2703, 2705, 2707, 2709, 2711, 2713, 2715, 2717, 2719, 2721, 2723, 2725, 2727, 2729, 2731, 2733

Table IB: Nucleic acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs
1	1	FR_OEX	B0124	E. coli	38	non-targeted	-
1	2	FR_OEX	B0730	E. coli	146	non-targeted	-
1	3	FR_OEX	B1528	E. coli	165	non-targeted	-
1	4	FR_OEX	B2148	E. coli	242	non-targeted	-
1	5	FR_OEX	B2573	E. coli	312	non-targeted	-
1	6	FR_OEX	B2600	E. coli	468	plastidic	-
1	7	FR_OEX	B2699	E. coli	534	non-targeted	938, 940, 942, 944, 946, 948, 950, 952, 954
1	8	FR_OEX	B2764	E. coli	970	non-targeted	1120, 1122, 1124, 1126, 1128, 1130
1	9	FR_OEX	B3262	E. coli	1143	non-targeted	-
1	10	FR_OEX	B3363	E. coli	1174	non-targeted	1488, 1490, 1492, 1494, 1496, 1498, 1500, 1502, 1504, 1506, 1508, 1510, 1512
1	11	FR_OEX	B3644	E. coli	1521	non-targeted	-
1	12	FR_OEX	YCR087W	S. cerevis iae	1660	non-targeted	-
1	13	FR_OEX	YGR150C	S. cerevis iae	1664	non-targeted	-
1	14	FR_OEX	YIL172C	S. cerevis iae	1691	non-targeted	-

1.		2.		3.		4.		5.		6.		7.		
Appli- cation	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Nucleic Acid Homologs							
	1	15	FR_OEX	YLR168C	S. cerevis iae	1859	non-targeted	1895						
	1	16	FR_OEX	YLR407W	S. cerevis iae	1904	non-targeted	-						
	1	17	FR_OEX	YNL241C	S. cerevis iae	1920	plastidic	2410, 2412, 2414, 2416, 2418, 2420, 2422, 2424, 2426, 2428, 2430, 2432, 2434, 2436, 2438, 2440, 2442, 2444, 2446						
	1	18	FR_OEX	YOR260W	S. cerevis iae	2455	non-targeted	-						
	1	19	FR_OEX	YOR299W	S. cerevis iae	2477	non-targeted	-						
	1	20	FR_OEX	YPL079W	S. cerevis iae	2507	non-targeted	2637, 2639, 2641, 2643, 2645, 2647, 2649, 2651, 2653, 2655, 2657, 2659, 2661, 2663, 2665, 2667, 2669, 2671, 2673, 2675						
	1	21	FR_OEX	YPL151C	S. cerevis iae	2683	non-targeted	2735, 2737, 2739, 2741, 2743						

Table IIA: Amino acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
1	1	FR_OEX	B0124	E. coli	39	non-targeted	41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129
1	2	FR_OEX	B0730	E. coli	147	non-targeted	149, 151, 153, 155, 157
1	3	FR_OEX	B1528	E. coli	166	non-targeted	168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232
1	4	FR_OEX	B2148	E. coli	243	non-targeted	245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303
1	5	FR_OEX	B2573	E. coli	313	non-targeted	315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461
1	6	FR_OEX	B2600	E. coli	469	plastidic	471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523
1	7	FR_OEX	B2699	E. coli	535	non-targeted	537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1231, 1233, 1235, 1237, 1239, 1241, 1243, 1245, 1247, 1249, 1251, 1253, 1255, 1257, 1259, 1261, 1263, 1265, 1267, 1269, 1271, 1273, 1275, 1277, 1279, 1281, 1283, 1285, 1287, 1289, 1291, 1293, 1295, 1297, 1299, 1301, 1303

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
							589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937
1	8	FR_OEX	B2764	E. coli	971	non-targeted	973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119
1	9	FR_OEX	B3262	E. coli	1144	non-targeted	1146, 1148, 1150, 1152, 1154, 1156, 1158, 1160, 1162, 1164

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
1	10	FR_OEX	B3363	E. coli	1175	non-targeted	1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1231, 1233, 1235, 1237, 1239, 1241, 1243, 1245, 1247, 1249, 1251, 1253, 1255, 1257, 1259, 1261, 1263, 1265, 1267, 1269, 1271, 1273, 1275, 1277, 1279, 1281, 1283, 1285, 1287, 1289, 1291, 1293, 1295, 1297, 1299, 1301, 1303, 1305, 1307, 1309, 1311, 1313, 1315, 1317, 1319, 1321, 1323, 1325, 1327, 1329, 1331, 1333, 1335, 1337, 1339, 1341, 1343, 1345, 1347, 1349, 1351, 1353, 1355, 1357, 1359, 1361, 1363, 1365, 1367, 1369, 1371, 1373, 1375, 1377, 1379, 1381, 1383, 1385, 1387, 1389, 1391, 1393, 1395, 1397, 1399, 1401, 1403, 1405, 1407, 1409, 1411, 1413, 1415, 1417, 1419, 1421, 1423, 1425, 1427, 1429, 1431, 1433, 1435, 1437, 1439, 1441, 1443, 1445, 1447, 1449, 1451, 1453, 1455, 1457, 1459, 1461, 1463, 1465, 1467, 1469, 1471, 1473, 1475, 1477, 1479, 1481, 1483, 1485, 1487
	11	FR_OEX	B3644	E. coli	1522	non-targeted	1524, 1526, 1528, 1530, 1532, 1534, 1536, 1538, 1540, 1542, 1544, 1546, 1548, 1550, 1552, 1554, 1556, 1558, 1560, 1562, 1564, 1566, 1568, 1570, 1572, 1574, 1576, 1578, 1580, 1582, 1584, 1586, 1588, 1590, 1592, 1594, 1596, 1598, 1600, 1602, 1604, 1606, 1608, 1610, 1612, 1614, 1616, 1618, 1620, 1622, 1624, 1626, 1628, 1630, 1632, 1634, 1636, 1638, 1640, 1642, 1644, 1646, 1648, 1650, 1652

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
1	12	FR_OEX	YCR087W	S. cerevis iae	1661	non-targeted	-
1	13	FR_OEX	YGR150C	S. cerevis iae	1665	non-targeted	1667, 1669, 1671, 1673
1	14	FR_OEX	YIL172C	S. cerevis iae	1692	non-targeted	1694, 1696, 1698, 1700, 1702, 1704, 1706, 1708, 1710, 1712, 1714, 1716, 1718, 1720, 1722, 1724, 1726, 1728, 1730, 1732, 1734, 1736, 1738, 1740, 1742, 1744, 1746, 1748, 1750, 1752, 1754, 1756, 1758, 1760, 1762, 1764, 1766, 1768, 1770, 1772, 1774, 1776, 1778, 1780, 1782, 1784, 1786, 1788, 1790, 1792, 1794, 1796, 1798, 1800, 1802, 1804, 1806, 1808, 1810, 1812, 1814, 1816, 1818, 1820, 1822, 1824, 1826, 1828, 1830, 1832, 1834, 1836, 1838, 1840, 1842, 1844, 1846, 1848, 1850
1	15	FR_OEX	YLR168C	S. cerevis iae	1860	non-targeted	1862, 1864, 1866, 1868, 1870, 1872, 1874, 1876, 1878, 1880, 1882, 1884, 1886, 1888, 1890, 1892, 1894
1	16	FR_OEX	YLR407W	S. cerevis iae	1905	non-targeted	1907, 1909, 1911
1	17	FR_OEX	YNL241C	S. cerevis iae	1921	plastidic	1923, 1925, 1927, 1929, 1931, 1933, 1935, 1937, 1939, 1941, 1943, 1945, 1947, 1949, 1951, 1953, 1955, 1957, 1959, 1961, 1963, 1965, 1967, 1969, 1971, 1973, 1975, 1977, 1979, 1981,

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
							1983, 1985, 1987, 1989, 1991, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, 2017, 2019, 2021, 2023, 2025, 2027, 2029, 2031, 2033, 2035, 2037, 2039, 2041, 2043, 2045, 2047, 2049, 2051, 2053, 2055, 2057, 2059, 2061, 2063, 2065, 2067, 2069, 2071, 2073, 2075, 2077, 2079, 2081, 2083, 2085, 2087, 2089, 2091, 2093, 2095, 2097, 2099, 2101, 2103, 2105, 2107, 2109, 2111, 2113, 2115, 2117, 2119, 2121, 2123, 2125, 2127, 2129, 2131, 2133, 2135, 2137, 2139, 2141, 2143, 2145, 2147, 2149, 2151, 2153, 2155, 2157, 2159, 2161, 2163, 2165, 2167, 2169, 2171, 2173, 2175, 2177, 2179, 2181, 2183, 2185, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2201, 2203, 2205, 2207, 2209, 2211, 2213, 2215, 2217, 2219, 2221, 2223, 2225, 2227, 2229, 2231, 2233, 2235, 2237, 2239, 2241, 2243, 2245, 2247, 2249, 2251, 2253, 2255, 2257, 2259, 2261, 2263, 2265, 2267, 2269, 2271, 2273, 2275, 2277, 2279, 2281, 2283, 2285, 2287, 2289, 2291, 2293, 2295, 2297, 2299, 2301, 2303, 2305, 2307, 2309, 2311, 2313, 2315, 2317, 2319, 2321, 2323, 2325, 2327, 2329, 2331, 2333, 2335, 2337, 2339, 2341, 2343, 2345, 2347, 2349, 2351, 2353, 2355, 2357, 2359, 2361, 2363, 2365, 2367, 2369, 2371, 2373, 2375, 2377, 2379, 2381, 2383, 2385, 2387, 2389, 2391, 2393, 2395, 2397, 2399, 2401, 2403, 2405, 2407, 2409
1	18	FR_OEX	YOR260W	S.	2456	non-targeted	2458, 2460, 2462, 2464, 2466

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
				cerevis iae			
1	19	FR_OEX	YOR299W	S. cerevis iae	2478	non-targeted	2480, 2482, 2484, 2486, 2488, 2490, 2492
1	20	FR_OEX	YPL079W	S. cerevis iae	2508	non-targeted	2510, 2512, 2514, 2516, 2518, 2520, 2522, 2524, 2526, 2528, 2530, 2532, 2534, 2536, 2538, 2540, 2542, 2544, 2546, 2548, 2550, 2552, 2554, 2556, 2558, 2560, 2562, 2564, 2566, 2568, 2570, 2572, 2574, 2576, 2578, 2580, 2582, 2584, 2586, 2588, 2590, 2592, 2594, 2596, 2598, 2600, 2602, 2604, 2606, 2608, 2610, 2612, 2614, 2616, 2618, 2620, 2622, 2624, 2626, 2628, 2630, 2632, 2634, 2636
1	21	FR_OEX	YPL151C	S. cerevis iae	2684	non-targeted	2686, 2688, 2690, 2692, 2694, 2696, 2698, 2700, 2702, 2704, 2706, 2708, 2710, 2712, 2714, 2716, 2718, 2720, 2722, 2724, 2726, 2728, 2730, 2732, 2734

Table IIB: Amino acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs
1	1	FR_OEX	B0124	E. coli	39	non-targeted	-
1	2	FR_OEX	B0730	E. coli	147	non-targeted	-
1	3	FR_OEX	B1528	E. coli	166	non-targeted	-
1	4	FR_OEX	B2148	E. coli	243	non-targeted	-
1	5	FR_OEX	B2573	E. coli	313	non-targeted	-
1	6	FR_OEX	B2600	E. coli	469	plastidic	-
1	7	FR_OEX	B2699	E. coli	535	non-targeted	939, 941, 943, 945, 947, 949, 951, 953, 955
1	8	FR_OEX	B2764	E. coli	971	non-targeted	1121, 1123, 1125, 1127, 1129, 1131
1	9	FR_OEX	B3262	E. coli	1144	non-targeted	-
1	10	FR_OEX	B3363	E. coli	1175	non-targeted	1489, 1491, 1493, 1495, 1497, 1499, 1501, 1503, 1505, 1507, 1509, 1511, 1513
1	11	FR_OEX	B3644	E. coli	1522	non-targeted	-
1	12	FR_OEX	YCR087W	S. cerevis iae	1661	non-targeted	-
1	13	FR_OEX	YGR150C	S. cerevis iae	1665	non-targeted	-
1	14	FR_OEX	YIL172C	S. cerevis iae	1692	non-targeted	-

1.		2.		3.		4.		5.		6.		7.		
Appli- cation	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Polypeptide Homologs							
	1	15	FR_OEX	YLR168C	S. cerevis iae	1860	non-targeted	1896						
	1	16	FR_OEX	YLR407W	S. cerevis iae	1905	non-targeted	-						
	1	17	FR_OEX	YNL241C	S. cerevis iae	1921	plastidic	2411, 2413, 2415, 2417, 2419, 2421, 2423, 2425, 2427, 2429, 2431, 2433, 2435, 2437, 2439, 2441, 2443, 2445, 2447						
	1	18	FR_OEX	YOR260W	S. cerevis iae	2456	non-targeted	-						
	1	19	FR_OEX	YOR299W	S. cerevis iae	2478	non-targeted	-						
	1	20	FR_OEX	YPL079W	S. cerevis iae	2508	non-targeted	2638, 2640, 2642, 2644, 2646, 2648, 2650, 2652, 2654, 2656, 2658, 2660, 2662, 2664, 2666, 2668, 2670, 2672, 2674, 2676						
	1	21	FR_OEX	YPL151C	S. cerevis iae	2684	non-targeted	2736, 2738, 2740, 2742, 2744						

Table III: Primer nucleic acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Primers
1	1	FR_OEX	B0124	E. coli	38	non-targeted	130, 131
1	2	FR_OEX	B0730	E. coli	146	non-targeted	158, 159
1	3	FR_OEX	B1528	E. coli	165	non-targeted	233, 234
1	4	FR_OEX	B2148	E. coli	242	non-targeted	304, 305
1	5	FR_OEX	B2573	E. coli	312	non-targeted	462, 463
1	6	FR_OEX	B2600	E. coli	468	plastidic	524, 525
1	7	FR_OEX	B2699	E. coli	534	non-targeted	956, 957
1	8	FR_OEX	B2764	E. coli	970	non-targeted	1132, 1133
1	9	FR_OEX	B3262	E. coli	1143	non-targeted	1165, 1166
1	10	FR_OEX	B3363	E. coli	1174	non-targeted	1514, 1515
1	11	FR_OEX	B3644	E. coli	1521	non-targeted	1653, 1654
1	12	FR_OEX	YCR087W	S. cerevis iae	1660	non-targeted	1662, 1663
1	13	FR_OEX	YGR150C	S. cerevis iae	1664	non-targeted	1674, 1675
1	14	FR_OEX	YIL172C	S. cerevis iae	1691	non-targeted	1851, 1852
1	15	FR_OEX	YLR168C	S.	1859	non-targeted	1897, 1898

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Primers
				cerevis iae			
1	16	FR_OEX	YLR407W	S. cerevis iae	1904	non-targeted	1912, 1913
1	17	FR_OEX	YNL241C	S. cerevis iae	1920	plastidic	2448, 2449
1	18	FR_OEX	YOR260W	S. cerevis iae	2455	non-targeted	2467, 2468
1	19	FR_OEX	YOR299W	S. cerevis iae	2477	non-targeted	2493, 2494
1	20	FR_OEX	YPL079W	S. cerevis iae	2507	non-targeted	2677, 2678
1	21	FR_OEX	YPL151C	S. cerevis iae	2683	non-targeted	2745, 2746

Table IV: Consensus amino acid sequence ID numbers

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Consensus / Pattern Sequences
1	1	FR_OEX	B0124	E. coli	39	non-targeted	132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145
1	2	FR_OEX	B0730	E. coli	147	non-targeted	160, 161, 162, 163, 164
1	3	FR_OEX	B1528	E. coli	166	non-targeted	235, 236, 237, 238, 239, 240, 241
1	4	FR_OEX	B2148	E. coli	243	non-targeted	306, 307, 308, 309, 310, 311
1	5	FR_OEX	B2573	E. coli	313	non-targeted	464, 465, 466, 467
1	6	FR_OEX	B2600	E. coli	469	plastidic	526, 527, 528, 529, 530, 531, 532, 533
1	7	FR_OEX	B2699	E. coli	535	non-targeted	958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969
1	8	FR_OEX	B2764	E. coli	971	non-targeted	1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142
1	9	FR_OEX	B3262	E. coli	1144	non-targeted	1167, 1168, 1169, 1170, 1171, 1172, 1173
1	10	FR_OEX	B3363	E. coli	1175	non-targeted	1516, 1517, 1518, 1519, 1520
1	11	FR_OEX	B3644	E. coli	1522	non-targeted	1655, 1656, 1657, 1658, 1659
1	12	FR_OEX	YCR087W	S. cerevis iae	1661	non-targeted	-
1	13	FR_OEX	YGR150C	S. cerevis iae	1665	non-targeted	1676, 1677, 1678, 1679, 1680, 1681, 1682, 1683, 1684, 1685, 1686, 1687, 1688, 1689, 1690
1	14	FR_OEX	YIL172C	S. cerevis iae	1692	non-targeted	1853, 1854, 1855, 1856, 1857, 1858

Appli- cation	1.	2.	3.	4.	5.	6.	7.
	Hit	Project	Locus	Orga- nism	Lead SEQ ID	Target	SEQ IDs of Consensus / Pattern Sequences
1	15	FR_OEX	YLR168C	S. cerevis iae	1860	non-targeted	1899, 1900, 1901, 1902, 1903
1	16	FR_OEX	YLR407W	S. cerevis iae	1905	non-targeted	1914, 1915, 1916, 1917, 1918, 1919
1	17	FR_OEX	YNL241C	S. cerevis iae	1921	plastidic	2450, 2451, 2452, 2453, 2454
1	18	FR_OEX	YOR260W	S. cerevis iae	2456	non-targeted	2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476
1	19	FR_OEX	YOR299W	S. cerevis iae	2478	non-targeted	2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506
1	20	FR_OEX	YPL079W	S. cerevis iae	2508	non-targeted	2679, 2680, 2681, 2682
1	21	FR_OEX	YPL151C	S. cerevis iae	2684	non-targeted	2747, 2748

CLAIMS

1. A method for producing a transgenic plant cell, a plant or a part thereof with increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control, wherein the method comprises the steps of:
- 5 a) introducing into a plant cell a polynucleotide encoding a "disease resistance conferring protein" BSRP from Escherichia and/or yeast
- 10 b) generating from the plant cell the transgenic plant expressing the polynucleotide.
2. A method according to claim 1, wherein said BSRP has an activity selected from the group consisting of:
- 15 chorismate mutase-T and prephenate dehydrogenase, b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit,
- 20 transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein..
- 25 3. A method for producing a transgenic plant cell, a plant or a part thereof with increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control by increasing or generating one or more activities selected from the group consisting of:
- 30 chorismate mutase-T and prephenate dehydrogenase, b3262-protein, b3644-protein, chloramphenicol resistance protein homolog ydeA, Gamma subunit of the translation initiation factor eIF2B, glucose dehydrogenase, glucose-6-phosphate 1-dehydrogenase, peptidyl-prolyl cis-trans isomerase A (rotamase A), recombinase A, ribosomal protein, RNA polymerase sigma-E factor (sigma-24), Splicing factor that is found in the Cef1p subcomplex of the spliceosome (Prp46p), sulfite reductase (NADPH), flavoprotein beta subunit,
- 35 transcription regulator farR, fatty acyl- responsive, transmembrane pore-generating protein mglC, YCR087W-protein, YGR150c-protein, YIL172C-protein, YLR168C-protein, YLR407W-protein, and YOR299W-protein..

4. A method according to claim 3 wherein the activity of at least one polypeptide comprising a polypeptide selected from the group consisting of:
- 5 (i) a polypeptide comprising a polypeptide, a consensus sequence or at least one polypeptide motif as depicted in column 5 or 7 of Table II or of Table IV, respectively; or
- (ii) an expression product of a nucleic acid molecule comprising a polynucleotide as depicted in column 5 or 7 of Table I,
- (iii) or a functional equivalent of (i) or (ii);
- 10 is increased or generated.
5. A method of any one of the claims 1 or 3 wherein the expression of at least one nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of:
- 15 (a) a nucleic acid molecule encoding the polypeptide shown in column 5 or 7 of Table II or comprising a polypeptide as shown in column 5 or 7 of Table II;
- (b) a nucleic acid molecule shown in column 5 or 7 of Table I;
- 20 (c) a nucleic acid molecule, which, as a result of the degeneracy of the genetic code, can be derived from a polypeptide sequence depicted in column 5 or 7 of Table II and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control, a plant or a part thereof ;
- 25 (d) a nucleic acid molecule having at least 30 % identity with the nucleic acid molecule sequence of a polynucleotide comprising the nucleic acid molecule shown in column 5 or 7 of Table I and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a
- 30 corresponding non-transformed wild type control, a plant or a part thereof ;
- (e) a nucleic acid molecule encoding a polypeptide having at least 30 % identity with the amino acid sequence of the polypeptide encoded by the nucleic acid molecule of (a) to (c)
- 35 and having the activity represented by a polypeptide comprising a polynucleotide as depicted in column 5 of Table II and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a

corresponding non-transformed wild type control plant cell, a plant or a part thereof

- 5 (f) nucleic acid molecule which hybridizes with a nucleic acid molecule of (a) to (c) under stringent hybridization conditions and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 10 (g) a nucleic acid molecule encoding a polypeptide which can be isolated with the aid of monoclonal or polyclonal antibodies made against a polypeptide encoded by one of the nucleic acid molecules of (a) to (e) and having the activity represented by the polypeptide comprising a polypeptide as depicted in column 5 of Table I;
- 15 (h) a nucleic acid molecule encoding a polypeptide comprising the consensus sequence or one or more polypeptide motifs as shown in column 7 of Table IV and preferably having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II or IV;
- 20 (i) a nucleic acid molecule encoding a polypeptide having the activity represented by a protein as depicted in column 5 of Table II and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 25 (j) nucleic acid molecule which comprises a polynucleotide, which is obtained by amplifying a cDNA library or a genomic library using the primers in column 7 of Table III and preferably having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II, and
- 30 (k) a nucleic acid molecule which is obtainable by screening a suitable nucleic acid library under stringent hybridization conditions with a probe comprising a complementary sequence of a nucleic acid molecule of (a) or (b) or with a fragment thereof, having at least 15 nt, preferably 20 nt, 30 nt, 50 nt, 100 nt, 200 nt or 500 nt of a nucleic acid molecule complementary to a nucleic acid molecule sequence characterized in (a) to (e) and encoding a
- 35

polypeptide having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II; is increased or generated.

- 5 6. A method of any one of the claims 1 or 3 wherein the transgenic plant cell, plant or part thereof with increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control is derived from a monocotyledonous plant.
- 10 7. A method of any one of the claims 1 or 3 wherein the transgenic plant cell, plant or part thereof with increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control is derived from a dicotyledonous plant.
- 15 8. A method of any one of the claims 1 or 3 wherein the plant is selected from the group consisting of maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, oil seed rape, including canola and winter oil seed rape, corn, manihot, pepper, sunflower, flax, borage, safflower, linseed, primrose, rapeseed, turnip rape, tagetes, solanaceous plants, potato, tobacco, eggplant, tomato, Vicia species, pea, alfalfa, coffee, cacao, tea, Salix species, oil palm, coconut, perennial grass, forage crops and Arabidopsis thaliana.
- 20
- 25 9. A method of any one of the claims 1 or 3 wherein the transgenic plant cell, plant or part thereof with increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control is derived from a gymnosperm plant, preferably spruce, pine and fir.
- 30 10. An isolated nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of:
- (a) a nucleic acid molecule encoding the polypeptide shown in column 7 of Table II B;
- (b) a nucleic acid molecule shown in column 7 of Table I B;
- 35 (c) a nucleic acid molecule, which, as a result of the degeneracy of the genetic code, can be derived from a polypeptide sequence depicted in column 5 or 7 of Table II and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a

corresponding non-transformed wild type control plant cell, a plant or a part thereof ;

- 5 (d) a nucleic acid molecule having at least 30 % identity with the nucleic acid molecule sequence of a polynucleotide comprising the nucleic acid molecule shown in column 5 or 7 of Table I and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof ;
- 10 (e) a nucleic acid molecule encoding a polypeptide having at least 30 % identity with the amino acid sequence of the polypeptide encoded by the nucleic acid molecule of (a) to (c) and having the activity represented by a nucleic acid molecule comprising a polynucleotide as depicted in column 5 of Table I and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a
- 15 corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- (f) nucleic acid molecule which hybridizes with a nucleic acid molecule of (a) to (c) under stringent hybridization conditions and confers increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control plant
- 20 cell, a plant or a part thereof;
- (g) a nucleic acid molecule encoding a polypeptide which can be isolated with the aid of monoclonal or polyclonal antibodies made against a polypeptide encoded by one of the nucleic acid molecules of (a) to (e) and having the activity represented by the nucleic acid molecule comprising a polynucleotide as depicted in column 5 of
- 25 Table I;
- (h) a nucleic acid molecule encoding a polypeptide comprising the consensus sequence or one or more polypeptide motifs as shown in column 7 of Table IV and preferably having the activity represented by a protein comprising a polypeptide as depicted in column 5 of
- 30 Table II or IV;
- (i) a nucleic acid molecule encoding a polypeptide having the activity represented by a protein as depicted in column 5 of Table II and confers an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control plant cell, a plant or a part thereof;
- 35 (j) nucleic acid molecule which comprises a polynucleotide, which is obtained by amplifying a cDNA library or a genomic library using the primers in column 7 of Table III and preferably having the activity
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represented by a protein comprising a polypeptide as depicted in column 5 of Table II or IV;

and

- 5 (k) a nucleic acid molecule which is obtainable by screening a suitable nucleic acid library under stringent hybridization conditions with a probe comprising a complementary sequence of a nucleic acid molecule of (a) or (b) or with a fragment thereof, having at least 15 nt, preferably 20 nt, 30 nt, 50 nt, 100 nt, 200 nt or 500 nt of a nucleic acid molecule complementary to a nucleic acid molecule sequence
10 characterized in (a) to (e) and encoding a polypeptide having the activity represented by a protein comprising a polypeptide as depicted in column 5 of Table II;

- 15 whereby the nucleic acid molecule according to (a) to (j) is at least in one or more nucleotides different from the sequence depicted in column 5 or 7 of Table I A and preferably which encodes a protein which differs at least in one or more amino acids from the protein sequences depicted in column 5 or 7 of Table II A.

- 20 11. A nucleic acid construct which confers the expression of said nucleic acid molecule of claim 10 or of a nucleic acid molecule encoding the polypeptide shown in column 5 of Table II, comprising one or more regulatory elements, whereby expression of the nucleic acid in a host cell results in increased biotic stress resistance, preferably to pathogenic fungi as compared to a corre-
25 sponding non-transformed wild type control.

- 30 12. A vector comprising the nucleic acid molecule as claimed in claim 10 or of a nucleic acid molecule encoding the polypeptide shown in column 5 of Table II or the nucleic acid construct of claim 11, whereby expression of said coding nucleic acid in a host cell results in increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control, preferably comprising
35 (i) a promoter regulating constitutive expression of an operably linked polynucleotide in a plant,
(ii) a promoter regulating tissue-specific expression of an operably linked polynucleotide in a plant or
(iii) a promoter regulating expression of an operably linked polynucleotide in syncytia site of a plant upon nematode infection or

(iii) a promoter regulating pathogen-inducible expression of an operably linked polynucleotide .

- 5 13. A host cell, which has been transformed stably or transiently with the vector as claimed in claim 12 or the nucleic acid molecule as claimed in claim 10 or of a nucleic acid molecule encoding the polypeptide shown in column 5 of Table II or the nucleic acid construct of claim 11 and which shows due to the transformation an increased biotic stress resistance, preferably to pathogenic fungi as compared to a corresponding non-transformed wild type control.

10
14. A plant tissue, propagation material, seed, harvested material or a plant comprising the host cell as claimed in claim 13.
- 15 15. Method according to any of the claims 1 to 9, whereby additionally abiotic stress resistance, preferably water deficiency resistance, more preferably drought resistance is conferred.
- 20 16. A host cell, plant tissue, propagation material, seed, harvested material or a plant which has been transformed stably or transiently with the vector as claimed in claim 12 or the nucleic acid molecule as claimed in claim 10 or of a nucleic acid molecule encoding the polypeptide shown in column 5 of Table II or the nucleic acid construct of claim 11 and which shows due to the transformation an increased biotic stress resistance, preferably to pathogenic fungi and abiotic stress resistance, preferably water deficiency resistance, more

25 preferably drought resistance as compared to a corresponding non-transformed wild type control.
- 30 17. Method according to any of the claims 1 to 9 and/or 15, whereby additionally an increase in the content of a fine chemical selected from the group consisting of:

35 3,4-dihydroxyphenylalanine (dopa), alanine, α -linolenic acid (c18:cis[9,12,15]3), α -linolenic acid, c18:3 (c9,c12,c15), α -linolenic acid, α -tocopherol, arginine, aspartic acid, beta-carotene, beta-sitosterol, campesterol, cerotic acid (c26:0), citrulline, coenzyme q10, coenzyme q9, ferulic acid, fructose, fumarate, fumaric acid, gamma-tocopherol/ beta-tocopherol/ 2,3-dimethyl-5-phytyl-quinol, gamma-tocopherol/beta-tocopherol, gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose, glutamate, glyceric acid, glycerol, glycine, hexadeca-dienoic acid (c16:cis

5 [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), homoserine, isoleucine, isopentenyl pyrophosphate, lacton of trihydroxybutyric acid, leucine, lignoceric acid (c24:0), linoleic acid (c18:cis[9,12]2), linoleic acid c18:2 (c9,c12), lutein, malic acid, methylgalactopyranosid, myo-inositol, palmitic acid (c16:0), phenylalanine, proline, putrescine, raffinose, salicylic acid, serine, shikimic acid, sinapic acid, stearic acid (c18:0), succinate, succinic acid, threonine, trihydroxybutanoic acid, tryptophane, tyrosine, valine and zeaxanthin,, is conferred.

10 18. A host cell, plant tissue, propagation material, seed, harvested material or a plant which has been transformed stably or transiently with the vector as claimed in claim 12 or the nucleic acid molecule as claimed in claim 10 or of a nucleic acid molecule encoding the polypeptide shown in column 5 of Table II or the nucleic acid construct of claim 11

15 and/or host cell, plant tissue, propagation material, seed, harvested material or a plant of claim 16

and which shows due to the transformation an an increase in the content of a fine chemical selected from the group consisting of:

20 3,4-dihydroxyphenylalanine (dopa), alanine, a-linolenic acid (c18:cis[9,12,15]3), a-linolenic acid, c18:3 (c9,c12,c15), alpha-linolenic acid, alpha-tocopherol, arginine, aspartic acid, beta-carotene, beta-sitosterol, campesterol, cerotic acid (c26:0), citrulline, coenzyme q10, coenzyme q9, ferulic acid, fructose, fumarate, fumaric acid, gamma-tocopherol/ beta-tocopherol/ 2,3-dimethyl-5-phytyl-quinol, gamma-tocopherol/beta-tocopherol,

25 gamma-tocopherol/beta-tocopherol/2,3-dimethyl-5-phytylquinol, glucose, glutamate, glyceric acid, glycerol, glycine, hexadeca-dienoic acid (c16:cis [7,10]2), hexadeca-trienoic acid (c16:cis [7,10,13]3), homoserine, isoleucine, isopentenyl pyrophosphate, lacton of trihydroxybutyric acid, leucine, lignoceric acid (c24:0), linoleic acid (c18:cis[9,12]2), linoleic acid c18:2 (c9,c12), lutein,

30 malic acid, methylgalactopyranosid, myo-inositol, palmitic acid (c16:0), phenylalanine, proline, putrescine, raffinose, salicylic acid, serine, shikimic acid, sinapic acid, stearic acid (c18:0), succinate, succinic acid, threonine, trihydroxybutanoic acid, tryptophane, tyrosine, valine and zeaxanthin, as compared to a corresponding non-transformed wild type control.

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Plasmid Figures

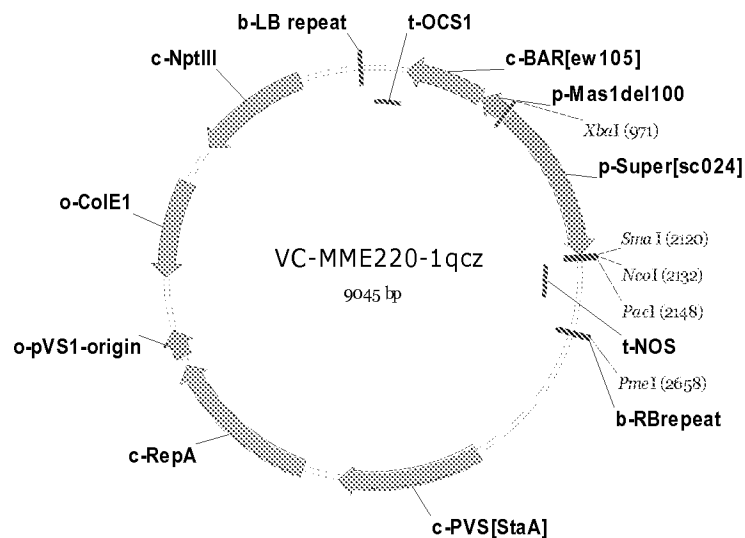


Fig. 1 Vector VC-MME220-1qcz (SEQ ID NO: 4) used for cloning gene of interest for non-targeted expression.

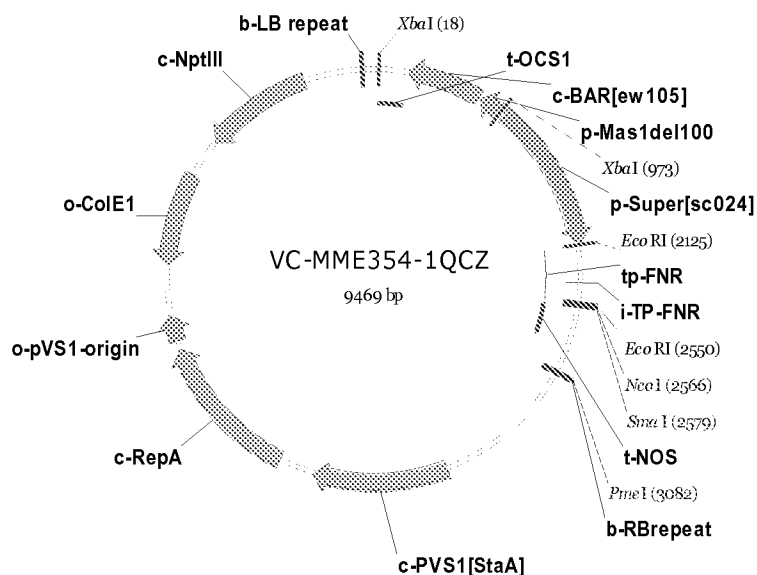


Fig. 2 Vector VC-MME354-1QCZ (SEQ ID NO: 2) used for cloning gene of interest for plastidic targeted expression.

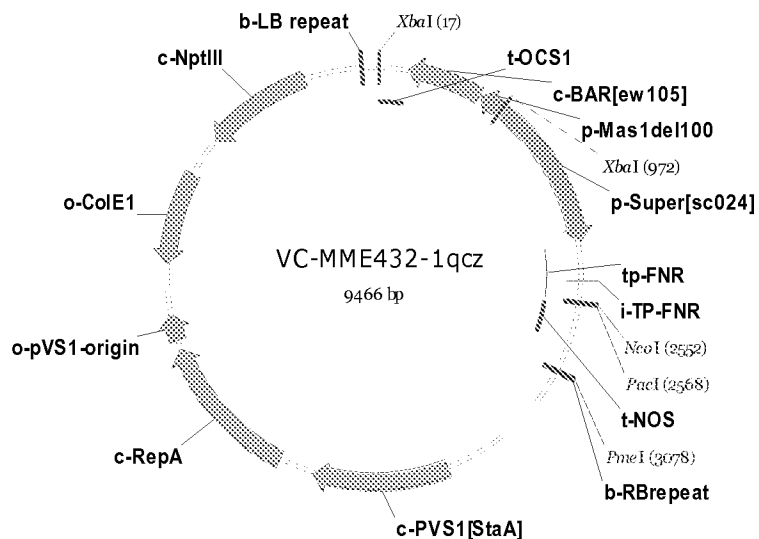


Fig. 3 Vector VC-MME432-1qcz (SEQ ID NO: 5) used for cloning gene of interest for plastidic targeted expression.

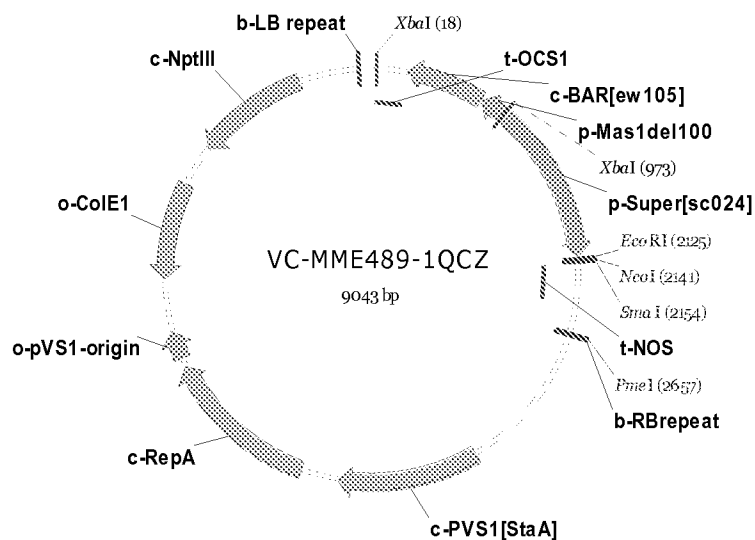


Fig. 4 Vector VC-MME489-1QCZ (SEQ ID NO: 7) used for cloning gene of interest for non-targeted expression and cloning of a targeting sequence.

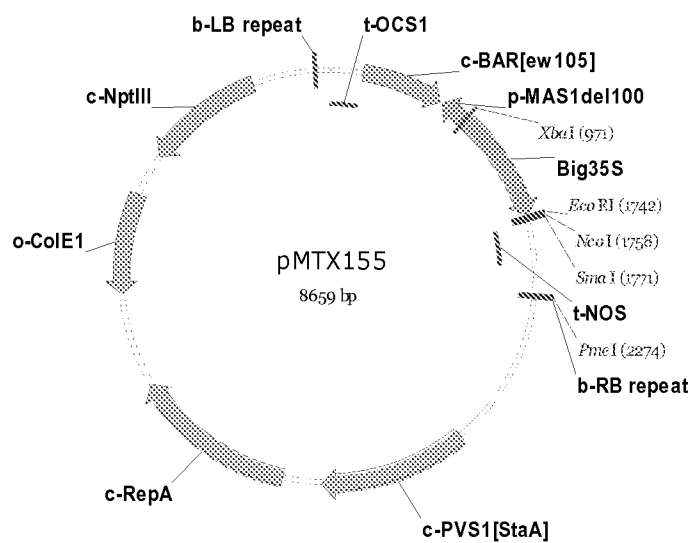


Fig. 5 Vector pMTX155 (SEQ ID NO: 1) used for used for cloning gene of interest for non-targeted expression.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/062533

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/82 A01H5/00 C12N15/52 C12N15/31 A01N63/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N A01H A01N

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

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

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/089561 A (MONSANTO TECHNOLOGY LLC [US]) 14 November 2002 (2002-11-14) page 37, line 20 - page 38, line 3; sequences 3,4	1-18
X	WO 02/00901 A (SUNGENE GMBH & CO KGAA [DE]; BADUR RALF [DE]; GEIGER MICHAEL [DE]; KUN) 3 January 2002 (2002-01-03) examples 1-3; sequence 1 2 ----- -/--	1-18



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

26 November 2009

Date of mailing of the international search report

12/02/2010

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2009/062533

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURPHY ALEX M ET AL: "A role for inositol hexakisphosphate in the maintenance of basal resistance to plant pathogens" PLANT JOURNAL, [Online] vol. 56, no. 4, 20 August 2008 (2008-08-20), pages 638-652, XP002557384 ISSN: 0960-7412 abstract	1
X	PRABHAVATHI V ET AL: "Mannitol-accumulating transgenic eggplants exhibit enhanced resistance to fungal wilts" PLANT SCIENCE (OXFORD), vol. 173, no. 1, July 2007 (2007-07), pages 50-54, XP002557385 ISSN: 0168-9452 abstract	1
X	US 6 018 106 A (HUNT ARTHUR G [US] ET AL) 25 January 2000 (2000-01-25) the whole document	1
X	WO 99/11806 A (UNIV RUTGERS [US]) 11 March 1999 (1999-03-11) the whole document	1
P,X	WO 2009/037279 A (BASF PLANT SCIENCE GMBH [DE]; PUZIO PIOTR [BE]; BLAESING OLIVER [DE];) 26 March 2009 (2009-03-26) sequences 2600,2601	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/062533

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see annex

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1 (claimed): claims 1-18 in so far as applicable

Method for producing a transgenic plant (cell etc) with increased biotic stress resistance by introducing a polynucleotide encoding a bifunctional chorismate mutase/prephenate dehydrogenase specifically as defined by SEQ ID NOS:468 and 469; methods based on said method; isolated nucleic acid molecule defined by reference to SEQ ID NO:468 which encodes SEQ ID NO: 469 with bifunctional chorismate mutase prephenate dehydrogenase activity; nucleic acid constructs, vector, host cell based on said sequences.

Inventions 2 to 1262 (claimed): claims 1-18 in so far as applicable

Methods for producing a transgenic plant (cell etc) with increased biotic stress resistance by introducing a polynucleotide (sequence) encoding a polypeptide (sequence) with the activity defined in the claims and by reference to the SEQ ID NOS in the tables I, II, III and IV mentioned in the claims; nucleic acid molecules encoding polypeptides as defined above; nucleic acid constructs, vector, hosts based on said sequences. Wherein each individual nucleic acid sequence (SEQ ID NO:) with its corresponding encoded polypeptide sequence (SEQ ID NO:) represents a single and independent claimed invention.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2009/062533

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02089561	A	14-11-2002	AT 419366 T	15-01-2009
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			BR 0209483 A	06-07-2004
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