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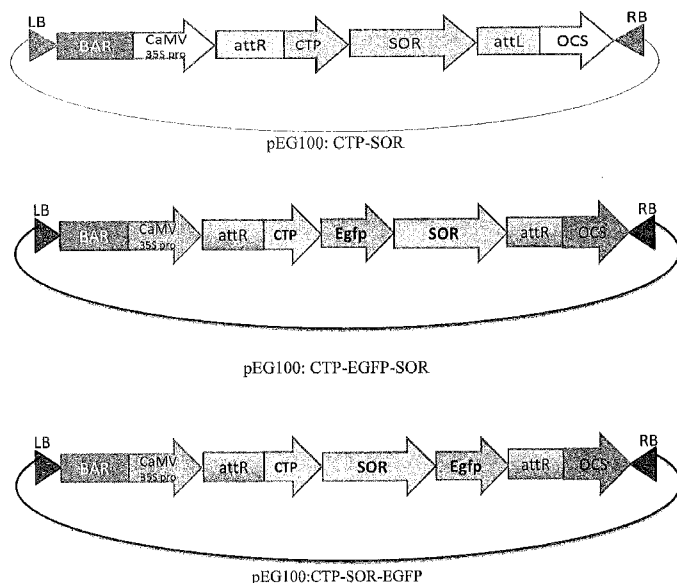
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[Continued on next page]

(54) Title: TRANSGENIC EXPRESSION OF ARCHAEA SUPEROXIDE REDUCTASE

Fig. 1.



(57) Abstract: This invention provides a stably transformed plant, plant part and/or plant cell, comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species, wherein said stably transformed plant, plant cell, and/or plant part has increased disease resistance. The invention further provides a method of increasing disease resistance in a plant, plant cell, or plant part, comprising: introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control. Additionally provided are plants, plant parts, and plant cells produced by the methods of the invention, as well as progeny and products produced therefrom.



— with sequence listing part of description (Rule 5.2(a))

TRANSGENIC EXPRESSION OF ARCHAEA SUPEROXIDE REDUCTASE

STATEMENT OF PRIORITY

5 This application claims the benefit, under 35 U.S.C. § 119 (e), of U.S. Provisional Application No. 61/838,817 was filed on June 24, 2013, the entire contents of which is incorporated by reference herein.

STATEMENT OF GOVERNMENT SUPPORT

10 This invention was supported in part by funding provided under Grant No DE-AR0000207 from the United States Department of Energy (DOE). The United States government has certain rights in this invention.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

15 A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5051-836WO_ST25.txt, 64,675 bytes in size, generated June 17, 2014 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated herein by reference into the specification for its disclosures.

FIELD OF THE INVENTION

20 The present invention relates to compositions and methods for expression of archaea superoxide reductase in a plant and other organisms by transforming the plant or other organism with a heterologous polynucleotide encoding a superoxide reductase from an archaeon species. The present invention further relates to methods and compositions for
25 increasing disease resistance in a plant.

BACKGROUND

Reactive oxygen species (ROS) are chemically reactive molecules formed due to incomplete reduction of oxygen and include superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide
30 (H_2O_2), and hydroxyl radicals (HO). ROS are highly reactive due to the presence of unpaired electrons. ROS are natural byproducts of normal metabolism of oxygen in many organisms and play an important role in cell signaling and homeostasis. However, elevated levels of ROS can have detrimental results. The levels of ROS can increase dramatically when an organism is exposed to various environmental stresses such as exposure to heat, excessive

light, drought, anoxia, toxins, pathogens, and the like, resulting in oxidative damage and cell death. In plants, for example, oxidative damage from excess ROS can result in reduced photosynthetic efficiency. Plants and other organisms have endogenous ROS metabolizing enzymes such as superoxide dismutase, catalase and peroxidase for preventing the buildup of ROS. However, these endogenous protective mechanisms can be insufficient when the organism experiences environmental stress conditions.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a stably transformed plant, plant part or plant cell, comprising a heterologous polynucleotide encoding a superoxide reductase from an archaeon species, wherein said stably transformed plant, plant part or plant cell has increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In some aspects, the superoxide reductase is localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, and/or as a membrane associated protein of said transformed plant, plant part or plant cell.

In another aspect of the invention, the present invention provides a stably transformed plant, plant part, plant cell, yeast cell or bacterial cell comprising a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin). In some aspects, the superoxide reductase is localized to the chloroplast, cytosolic membrane, peroxisome, cell wall, mitochondria, periplasm and/or as a membrane associated protein of said stably transformed plant, plant cell, plant part, yeast cell or bacterial cell.

In a further aspect, the present invention provides a method of increasing disease resistance in a plant, plant cell, or plant part, comprising: introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control. In some aspects, the stably transformed plant part or plant cell is regenerated into a stably transformed plant comprising in its genome the heterologous polynucleotide encoding a superoxide reductase from an archaeon species, thereby producing a plant having increased disease resistance as compared to a control.

In other aspects, the present invention provides progeny and crops produced from the stably transformed plants of the invention as well as products produced from the transformed plants, plant cells, plant parts, yeast cells and/or bacterial cells of this invention.

The foregoing and other objects and aspects of the present invention are explained in
5 detail in the drawings and specification set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows vector maps of constructs for plant transformation: pEG100: CTP-SOR, pEG100:CTP-EGFP-SOR and pEG100:CTP-SOR-EGFP.

10 **Figure 2** shows vector maps of constructs for yeast transformation: pFL36:SOR and pFL36: EGFP-SOR

Figure 3 shows images of leaves of wild type (WT) and transgenic plants challenged with powdery mildew spores.

DETAILED DESCRIPTION

15 This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Thus, the invention contemplates that in
20 some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not
25 to exhaustively specify all permutations, combinations and variations thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

30 All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions of equivalent techniques that would be apparent to one of

skill in the art.

Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as a dosage or time period and the like, refers to variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y" and phrases such as "from about X to Y" mean "from about X to about Y."

The terms "comprise," "comprises" and "comprising" as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

Archaea are single celled microorganisms many of which have developed the ability to survive in extreme environments such as high heat and salt (i.e., extremophiles).

Pyrococcus furiosus is an extremophilic (hyperthermophilic) species of archaea with

optimum growth at 100°C. It is found in hydrothermal vents and is a strict anaerobe at growth permissible temperatures. *P. furiosus* uses an enzyme, superoxide reductase (SOR), to deal with ROS. *P. furiosus* SOR has a functional temperature range of about 4°C to about 100°C. Unlike superoxide dismutase (SOD), which is an endogenous enzyme found in plants, including algae, and in yeast and aerobic bacteria, SOR is more efficient in removing ROS and does so without producing oxygen (thereby reducing the potential for further ROS generation). Notably, there is substantial similarity between SOR from *P. furiosus* and other archaea as well as from mesophilic bacteria. Non-limiting examples of additional archaea SOR useful with this invention include those listed in **Table 1**, below.

Table 1. Exemplary archaeal species useful with the invention

Archaeal Species	SOR Accession #	% Identity to <i>P. furiosus</i> SOR
<i>Thermococcus litoralis</i> DSM 5473	CP006670.1	97%
<i>Thermococcus sibiricus</i> MM 739	CP001463.1	88%
<i>Pyrococcus horikoshii</i> OT3	BA000001.2	74%
<i>Pyrococcus</i> sp. ST04	CP003534.1	73%
<i>Thermococcus nautili</i> strain 30-1	CP007264.1	73%
<i>Thermococcus</i> sp. AM4	CP002952.1	73%
<i>Pyrococcus yayanosii</i> CH1	CP002779.1	71%
<i>Thermococcus gammatolerans</i> EJ3	CP002670.1	70%
<i>Thermococcus onnurineus</i> NA1	CP000855.1	69%
<i>Candidatus Korarchaeum cryptofilum</i> OPF8	CP000968.1	69%
<i>Archaeoglobus fulgidus</i> DSM 4304	AE000782.1	68%
<i>Ferroglobus placidus</i> DSM 10642	CP001899.1	67%
<i>Thermococcus barophilus</i> MP	CP006965.1	66%
<i>Aciduliprofundum</i> sp. MAR08-339	CP003168.1	65%
<i>Archaeoglobus sulfaticallidus</i> PM70-1	CP005290.1	65%

Accordingly, the present invention is directed to transgenic plants, plant parts, and/or plant cells comprising a heterologous polynucleotide encoding a superoxide reductase from an archaeon species and methods of increasing resistance to disease in said transgenic plants, plant cells, and/or plant parts. Other aspects of the invention are directed to a stably transformed plant, plant cell, plant part, yeast cell or bacterial cell comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species and a heterologous polynucleotide encoding a CO₂ transporter, wherein the stably transformed plant has increased disease resistance, increased stress tolerance and increased yield.

Thus, a first aspect of the present invention provides a stably transformed plant, plant part and/or plant cell comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species, wherein said stably transformed plant, plant cell, and/or plant part has increased disease resistance as compared to a control (e.g., a plant, plant

part or plant cell that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). A second aspect of the present invention provides a stably transformed plant, plant part and/or plant cell comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species, wherein

5 when the stably transformed plant, plant cell, and/or plant part is exposed to abiotic stress conditions said stably transformed plant, plant cell, and/or plant part has increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell exposed to abiotic stress that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In a third aspect of the present invention provides a

10 stably transformed plant, plant part and/or plant cell comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species, wherein the stably transformed plant, plant cell, and/or plant part is first exposed to abiotic stress conditions, then removed from said abiotic stress conditions and has increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell that does not

15 comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In some embodiments, the superoxide reductase is localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, and/or as a membrane associated protein of said stably transformed plant, plant cell, and/or plant part. In particular embodiments, the archaeon superoxide reductase is not localized in the cytosol or

20 cytosolic membrane of a plant, plant part or plant cell. In further embodiments, the archaeon superoxide reductase is not localized in the cytosol and/or cytosolic membrane of a plant, plant part or plant cell when said plant, plant part or plant cell is from *Arabidopsis thaliana* and/or a higher plant.

In another aspect of the invention, a stably transformed plant, plant part, plant cell,

25 yeast cell or bacterial cell comprising a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin) is provided. In some embodiments, the superoxide reductase can be localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, and/or as a membrane associated protein of said

30 stably transformed plant, plant part, plant cell, yeast cell or bacterial cell. In particular embodiments, the archaeon superoxide reductase is not localized in the cytosol or cytosolic membrane of a plant, plant part or plant cell. In further embodiments, the archaeon superoxide reductase is not localized in the cytosol and/or cytosolic membrane of a plant, plant part or plant cell when said plant, plant part or plant cell is from *Arabidopsis thaliana*

and/or a higher plant. In still further embodiments, a stably transformed plant, plant part or plant cell comprising a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter can have increased disease resistance as compared to a control plant, plant part or plant cell that does not comprise said first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and said second heterologous polynucleotide encoding a CO₂ transporter. In other embodiments, a stably transformed plant, plant part or plant cell comprising a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter can have increased stress resistance or tolerance and/or increased yield as compared to a control plant, plant part or plant cell that does not comprise said first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and said second heterologous polynucleotide encoding a CO₂ transporter.

In a further aspect, the present invention provides a method of increasing disease resistance (e.g., decreasing disease symptoms, decreasing pathogen growth and reproduction) in a plant, plant cell, or plant part, comprising: introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In some aspects, the present invention provides a method of increasing disease resistance (e.g., decreasing disease symptoms, decreasing pathogen growth and reproduction) in a plant, plant cell, or plant part, comprising: introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part; and exposing said stably transformed plant, plant cell, and/or plant part to abiotic stress conditions, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell that is exposed to abiotic stress conditions and does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In a further aspect, the present invention provides a method of increasing disease resistance (e.g., decreasing disease symptoms, decreasing pathogen growth and reproduction) in a plant, plant cell, or plant part, comprising: introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to

produce a stably transformed plant, plant cell, or plant part; exposing said stably transformed plant, plant cell, and/or plant part to abiotic stress conditions; and removing the stably transformed plant, plant cell, and/or plant part from said abiotic stress conditions, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control (e.g., a plant, plant part or plant cell that is exposed to abiotic stress conditions, removed from abiotic stress conditions and does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species). In some embodiments, the archaeon superoxide reductase can be localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, and/or as a membrane associated protein of said stably transformed plant, plant cell, and/or plant part. In particular embodiments, the archaeon superoxide reductase is not localized in the cytosol or cytosolic membrane of a plant, plant part or plant cell. In further embodiments, the archaeon superoxide reductase is not localized in the cytosol and/or cytosolic membrane of a plant, plant part or plant cell when said plant, plant part or plant cell is from *Arabidopsis thaliana* and/or a higher plant. In still further embodiments, the method further comprises introducing into said stably transformed plant a heterologous polynucleotide encoding a CO₂ transporter.

In other aspects of the invention, a method of increasing stress resistance or tolerance and/or increasing yield in a plant in the presence of stress in a plant, plant cell, or plant part is provided, the method comprising: introducing into said plant, plant cell, or plant part a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased stress resistance or tolerance and/or increased yield in a plant in the presence of stress as compared to a control (e.g., a plant, plant part or plant cell that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species or said heterologous polynucleotide encoding a CO₂ transporter). As disclosed herein, the superoxide reductase can be localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, and/or as a membrane associated protein of said stably transformed plant, plant cell, and/or plant part. In particular embodiments, the archaeon superoxide reductase is not localized in the cytosol or cytosolic membrane of a plant, plant part or plant cell. In further embodiments, the archaeon superoxide reductase is not localized in the cytosol and/or cytosolic membrane of a plant, plant part or plant cell when said plant, plant part or plant cell is from *Arabidopsis thaliana* and/or a higher plant.

The terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof), as used herein, describe an elevation in, for example, the resistance of a plant, plant part or plant cell to disease. This increase can be observed by comparing the increase in the plant, plant part or plant cell transformed with the heterologous polynucleotide encoding said SOR to the appropriate control (e.g., a plant, plant part or plant cell lacking (i.e., not transformed with) the heterologous polynucleotide encoding said SOR from an archaeon species). Thus, as used herein, the terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof), and similar terms indicate an elevation of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 300%, 400%, 500% or more, or any range therein, as compared to a control (e.g., a plant, plant part, plant cell that does not comprise said heterologous polynucleotide encoding SOR from an archaeon species or a plant, plant part, plant cell, yeast cell, bacterial cell that does not comprise said heterologous polynucleotide encoding SOR from an archaeon species or said heterologous polynucleotide encoding a CO₂ transporter).

As used herein, the terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” “suppress,” and “decrease” (and grammatical variations thereof), describe, for example, a decrease in the disease observed in a plant, plant part, or plant cell comprising in its genome said heterologous polynucleotide encoding SOR from an archaeon species as compared to a control plant, plant part, or plant cell that does not comprise in its genome said heterologous polynucleotide encoding SOR from an archaeon species. Thus, as used herein, the terms “reduce,” “reduces,” “reduced,” “reduction,” “diminish,” “suppress,” and “decrease” and similar terms mean a decrease of at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, and the like, or more, or any range therein, as compared to a control (e.g., a plant, plant part, or plant cell that does not comprise in its genome said heterologous polynucleotide encoding SOR from an archaeon species or a plant, plant part, plant cell, yeast cell and/or bacterial cell that does not comprise said heterologous polynucleotide encoding SOR from an archaeon species and said heterologous polynucleotide encoding a CO₂ transporter).

As used herein, “increasing disease resistance” or “increased disease resistance” refers to, for example, decreasing disease symptoms on a plant in response to exposure to a pathogen, and/or decreasing the ability of a pathogen to survive, grow and/or reproduce on a

plant modified as described herein (e.g., a transgenic plant comprising a polynucleotide encoding SOR or a transgenic plant comprising a polynucleotide encoding SOR and a polynucleotide encoding a CO₂ transporter).

In some embodiments, an increase in disease resistance can mean a reduction in the size and in the number of disease lesions on the plant. Thus, in particular embodiments, a decrease in the number of lesions can be from about 10% to about 100%, from about 20% to about 90%, from about 30% to about 85%, from about 40% to about 80%, or from about 50% to about 75%. Thus, in some embodiments, a decrease in the number of lesions can be from about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or any range therein and the like. In other embodiments, the size of the lesion can be reduced by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or any range therein and the like.

As used herein, “increased stress resistance or stress tolerance” or “increasing stress resistance or stress tolerance” refers to the ability of a plant to be in the presence of an abiotic stress as defined herein resulting in a reduced affect of said abiotic stress on the plant’s growth, metabolism, yield and/or viability as compared to a control plant (e.g., a plant not comprising the heterologous polynucleotides encoding SOR and a CO₂ transporter as described herein).

“Yield” as used herein, refers to the amount (as measured by weight or number) of tissue produced per plant. Plant tissues can include any plant part (e.g., leaves, stems, stalks, seeds, fruits, and the like) or the whole plant itself.

As used herein, the terms “express,” “expresses,” “expressed” or “expression,” and the like, with respect to a nucleotide sequence (e.g., RNA or DNA) indicates that the nucleotide sequence is transcribed and, optionally, translated. Thus, a nucleotide sequence may express a polypeptide of interest or a functional untranslated RNA. A “functional” RNA includes any untranslated RNA that has a biological function in a cell, e.g., regulation of gene expression. Such functional RNAs include but are not limited to RNAi (e.g., siRNA, shRNA, antisense RNA), miRNA, ribozymes, RNA aptamers, and the like.

In some embodiments, the archaeon species can be a species from the genus *Pyrococcus*, a species from the genus *Thermococcus*, or a species from the genus *Archaeoglobus*. In other embodiments, the archaeon species can be *Pyrococcus furiosus* and the heterologous polynucleotide encoding a SOR can be a nucleotide sequence of **SEQ ID NO:1** or **SEQ ID NO:2**. In some embodiments, the heterologous polynucleotide encoding a SOR encodes an amino acid sequence of **SEQ ID NO:3** or **SEQ ID NO:4**. Thus, in some

embodiments, the invention provides a nucleotide sequence comprising, essentially consisting of, consisting of (a) a nucleotide sequence of **SEQ ID NO:2**; (b) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence of **SEQ ID NO:4**; and/or (c) a nucleotide sequence that differs from the nucleotide sequences of (a) or (b) above due to the degeneracy of the genetic code. In other embodiments, the invention provides an isolated polypeptide comprising, essentially consisting of or consisting of an amino acid sequence of **SEQ ID NO:4**.

In some embodiments, the heterologous polynucleotide encoding a SOR is operably associated with a targeting nucleotide sequence encoding a signal peptide that targets the heterologous SOR to the cytosol, cytosolic membrane (e.g., cytosolic surface of the plasma-membrane and other endogenous membranes including the nuclear envelope and endoplasmic reticulum), chloroplast, cell wall, peroxisome, mitochondria, periplasm and/or as a membrane associated protein. The signal sequence may be operably linked at the N- or C- terminus of the nucleic acid molecule. In some embodiments, the heterologous polynucleotide encoding a SOR is not operably associated with a targeting nucleotide sequence that encodes a signal peptide targeting said SOR to the cytosol and/or cytosolic membrane. In other embodiments, the heterologous polynucleotide encoding a SOR is not operably associated with a targeting nucleotide sequence that encodes a signal peptide targeting said SOR to the cytosolic membrane. In some particular embodiments, when the targeting nucleotide sequence encodes a signal peptide that targets the SOR to the cytosol and/or cytosolic membrane, the plant, plant part and/or plant cell is not from a higher plant. In other embodiments, when the targeting nucleotide sequence encodes a signal peptide that targets the SOR to the cytosol and/or cytosolic membrane, the plant, plant part and/or plant cell is not *Arabidopsis thaliana* or not from *Arabidopsis thaliana*.

Aquaporin is a high affinity CO₂ transporter with high similarity to the human CO₂ pore (AQP1) that has been identified in tobacco (NtAQP1, e.g., aquaporin) and shown to facilitate CO₂ membrane transport in plants (Uehlein et al. *Nature* **425**(6959): 734-7 (2003); Uehlein et al. *Plant Cell* **20**(3):648-57 (2008); Flexas et al. *Plant J.* **48**(3):427-39 (2006)). In some embodiments, a heterologous polynucleotide encoding a polypeptide having the activity of a CO₂/bicarbonate transporter can be used. Thus, in some embodiments, a heterologous polynucleotide encoding a polypeptide having the activity of a CO₂ transporter is from a plant (including, but not limited to, a saltwater algae), an extremophile archaea and/or extremophile bacteria (e.g. from the marine microalgae *Dunaliella* spp.; and/or *Hydrogenobacter thermophilis*).

In representative embodiments, a heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin) can comprise, consist essentially of or consist of a nucleotide sequence of **SEQ ID NO:58**, **SEQ ID NO:60** and/or **SEQ ID NO:62**, or a nucleotide sequence having substantial identity to said nucleotide sequences of **SEQ ID NO:58**, **SEQ ID NO:60** and/or **SEQ ID NO:62**. In other embodiments, an amino acid sequence of a CO₂ transporter can optionally comprise, consist essentially of or consist of the amino acid sequence of **SEQ ID NO:59**, **SEQ ID NO:61** and/or **SEQ ID NO:63**, or an amino acid sequence having substantial identity to said nucleotide sequences of the amino acid sequence of **SEQ ID NO:59**, **SEQ ID NO:61** and/or **SEQ ID NO:63**.

In particular embodiments, in addition to a heterologous polynucleotide encoding a SOR or a heterologous polynucleotide encoding a SOR and a heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin), a plant, plant part, plant cell, yeast or bacterial cell can further comprise an archaeal rubrerythrin reductase for conversion of hydrogen peroxide to water. Rubrerythrin reductase is an iron-dependent peroxidase that functions in vivo to remove the peroxide produced by superoxide reductase. Thus, a further embodiment of the invention includes a stably transformed plant comprising an expression cassette that comprises a SOR and a rubrerythrin reductase or an expression cassette that comprises SOR, CO₂ transporter and rubrerythrin reductase. In some embodiments, the SOR and rubrerythrin reductase are co-localized (i.e., they are expressed and targeted to the same or similar position in the transformed cell). In other embodiments, the SOR, CO₂ transporter, and rubrerythrin reductase are co-localized.

In some embodiments, an archaeal rubrerythrin reductase can be from *Pyrococcus furiosus*. In further embodiments, an archaeal rubrerythrin reductase can be encoded by the nucleotide sequence of **SEQ ID NO:50**. In still further embodiments, an archaeal rubrerythrin reductase can comprise, consist essentially of, or consist of the amino acid sequence of **SEQ ID NO:51**.

As discussed herein, the SOR, CO₂ transporter, and/or rubrerythrin reductase can be targeted to particular organelles. Thus, in some embodiments, the SOR, CO₂ transporter and/or rubrerythrin reductase polypeptides are in operable linkage or fused with a targeting or signal peptide (e.g., fusion protein) that directs the protein to the desired cellular location.

Signal peptides (and the targeting nucleotide sequences encoding them) are well known in the art and can be found in public databases such as the "Signal Peptide Website: An Information Platform for Signal Sequences and Signal Peptides." (www.signalpeptide.de); the "Signal Peptide Database"

(proline.bic.nus.edu.sg/spdb/index.html) (Choo et al., *BMC Bioinformatics* 6:249 (2005)(available on www.biomedcentral.com/1471-2105/6/249/abstract); ChloroP (www.cbs.dtu.dk/services/ChloroP/; predicts the presence of chloroplast transit peptides (cTP) in protein sequences and the location of potential cTP cleavage sites); LipoP (www.cbs.dtu.dk/services/LipoP/; predicts lipoproteins and signal peptides in Gram negative bacteria); MITOPROT (ihg2.helmholtz-muenchen.de/ihg/mitoprot.html; predicts mitochondrial targeting sequences); PlasMit (gecco.org.chemie.uni-frankfurt.de/plasmit/index.html; predicts mitochondrial transit peptides in *Plasmodium falciparum*); Predotar (urgi.versailles.inra.fr/predotar/predotar.html; predicts mitochondrial and plastid targeting sequences); PTS1 (mendel.imp.ac.at/mendeljsp/sat/pts1/PTS1predictor.jsp; predicts peroxisomal targeting signal 1 containing proteins); SignalP (www.cbs.dtu.dk/services/SignalP/; predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms: Gram-positive prokaryotes, Gram-negative prokaryotes, and eukaryotes). The SignalP method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks and hidden Markov models; and TargetP (www.cbs.dtu.dk/services/TargetP/); predicts the subcellular location of eukaryotic proteins - the location assignment is based on the predicted presence of any of the N-terminal presequences: chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP) or secretory pathway signal peptide (SP)). (See also, von Heijne, G., *Eur J Biochem* **133** (1) 17-21 (1983); Martoglio et al. *Trends Cell Biol* **8** (10):410-5 (1998); Hegde et al. *Trends Biochem Sci* **31**(10):563-71 (2006); Dultz et al. *J Biol Chem* **283**(15):9966-76 (2008); Emanuelsson et al. *Nature Protocols* **2**(4) 953-971(2007); Zuegge et al. **280**(1-2):19-26 (2001); Neuberger et al. *J Mol Biol.* **328**(3):567-79 (2003); and Neuberger et al. *J Mol Biol.* **328**(3):581-92 (2003)).

Exemplary signal peptides include, but are not limited to those provided in **Table 2**.

Table 2. Amino acid sequences of representative signal peptides.

Source	Sequence	Target
Rubisco small subunit (tobacco)	MASSVLSSAAVATRSNVAQANMVAPFTGLKSAASFPVSR KQLDITSIASNGGRVQC (SEQ ID NO:5)	chloroplast
<i>Saccharomyces cerevisiae</i> cox4	MLSLRQSIRFFKPATRTLCSRYLL (SEQ ID NO:7)	mitochondria
Arabidopsis aconitase	MYLTASSSASSSIIRAASSRSSLFSFRSVLSPSVSSTSPSSLL ARRSFGTISPAFRRWSHSFHSKPSPFRFTSQIRA (SEQ ID NO:9)	mitochondria
Yeast aconitase	MLSARSAIKRPIVRGLATV (SEQ ID NO:40)	mitochondria
Arabidopsis proline-rich protein 2 (AT2G21140)	MRILPKSGGGALCLLFVFALCSVAHS (SEQ ID NO:11)	cell wall/secretory pathway

PTS-2 (conserved in eukaryotes)	RLX ₅ HL (SEQ ID NO:13) MRLSIHAEHL (SEQ ID NO:14) SKL	peroxisome
Arabidopsis presequence protease1 (AT3G19170)	MLRTVSCLASRSSSSSLFFRFQFPRSYMSLTSSSTAALRVPSRNLRRISPSVAGRRLLLRRLRIPSAAVRSVNGQFSRLSVRA (SEQ ID NO:16)	mitochondria and chloroplast
<i>Chlamydomonas reinhardtii</i> -(Stroma-targeting cTPs: photosystem I (PSI) subunits P28, P30, P35 and P37, respectively)	MALVARPVLSARVAASRPVAARKAVRVSAKYGEN (SEQ ID NO:41) MQALSSRVNIAAKPQRAQLVVRAEEVKA (SEQ ID NO:42) MQTLASRPSLRASARVAPRRAPRVAVVTKAALDPQ (SEQ ID NO:43) MQALATRPASAIRPTKAARRSSVVVRADGFIG (SEQ ID NO:44)	chloroplast
<i>C. reinhardtii</i> – chlorophyll a/b protein (cabII-1)	MAFALASRKALQVTCKATGKKTAAKAAAPKSSGVEFYGPNAKWLGPYSEN (SEQ ID NO:45)	chloroplast
<i>C. reinhardtii</i> –Rubisco small subunit	MAAVIAKSSVSAAVARPARSSVRPMAALKPAVKAAPVAAPAQANQMMVWT (SEQ ID NO:46)	chloroplast
<i>C. reinhardtii</i> –ATPase-γ	MAAMLASKQGAFMGRSSFAPAPKGVASRGSQVAVGLKEV (SEQ ID NO:47)	chloroplast
<i>Escherichia coli</i> <i>phoA</i> alkaline phosphatase	MKQSTIAKAKKPLLFTPTVTKA (SEQ ID NO:48)	periplasm
<i>Arabidopsis thaliana</i> abscisic acid receptor PYL10	CVVQ (SEQ ID NO:34)	membrane

X₅ means any five amino acids can be present in the sequence to target the protein to the peroxisome (e.g. RLAVAVAHL, SEQ ID NO:49).

Thus, in representative embodiments of the invention, a heterologous polynucleotide
5 encoding a SOR and/or a heterologous polynucleotide encoding a CO₂ transporter to be expressed in a plant, plant cell, plant part can be operably linked to a chloroplast targeting sequence encoding a chloroplast signal peptide, optionally wherein said chloroplast signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of SEQ ID NO:5, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID
10 NO:44, SEQ ID NO:45, SEQ ID NO:46, or SEQ ID NO:47.

In other embodiments of the invention, a heterologous polynucleotide encoding a SOR and/or a heterologous polynucleotide encoding a CO₂ transporter to be expressed in a plant, plant part, plant cell or yeast cell can be operably linked to a mitochondrial targeting sequence encoding a mitochondrial signal peptide, optionally wherein said mitochondrial
15 signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:40.

In further embodiments, a heterologous polynucleotide encoding a SOR to be expressed in a plant, plant part, plant cell, yeast cell, or bacterial cell can be operably linked to a cell wall targeting sequence encoding a cell wall signal peptide, optionally wherein said

cell wall signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of **SEQ ID NO:11**.

In still further embodiments of the invention, a heterologous polynucleotide encoding a SOR to be expressed in a plant, plant part, plant cell, or a yeast cell can be operably linked to a peroxisomal targeting sequence encoding a peroxisomal signal peptide, optionally wherein said peroxisomal signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of **SEQ ID NO:13**, **SEQ ID NO:14**, or SKL.

In additional embodiments, a heterologous polynucleotide encoding a SOR to be expressed in a bacterial cell can be operably linked to a periplasmic targeting sequence encoding a periplasmic signal peptide, optionally wherein said periplasmic signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of **SEQ ID NO:48**.

In some embodiments, a heterologous polynucleotide encoding a SOR and/or a heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin) to be expressed in a plant, plant part, plant cell, yeast cell or bacterial cell can be operably linked to a membrane targeting sequence encoding a membrane signal peptide, optionally wherein said membrane signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of **SEQ ID NO:34**. In some embodiments, wherein when the heterologous polynucleotide encoding a SOR and/or a heterologous polynucleotide encoding a CO₂ transporter are targeted to a membrane, the SOR and/or the CO₂ transporter can be either linked directly to the membrane or to the membrane via a linkage to a membrane associated protein. In representative embodiments, a membrane associated protein includes but is not limited to the plasma membrane NADH oxidase (RbohA) (for *respiratory burst oxidase homolog A*) (Keller et al. *The Plant Cell Online* 10: 255-266 (1998)), annexin1 (ANN1) from *Arabidopsis thaliana* (Laohavisit et al. *Plant Cell Online* 24: 1522-1533 (2012)), and/or the nitrate transporter CHL1 (AtNRT1.1) (Tsay et al. "The Role of Plasma Membrane Nitrogen Transporters in Nitrogen Acquisition and Utilization," In, *The Plant Plasma Membrane* 19:223-236 Springer Berlin/Heidelberg (2011)).

Targeting to a membrane is similar to targeting to an organelle. Thus, specific sequences on a protein (targeting sequences or motifs) can be recognized by a transporter, which then imports the protein into an organelle or in the case of membrane association, the transporter can guide the protein to and associate it with a membrane. Thus, for example, a specific cysteine residue on a C-terminal motif of a protein can be modified posttranslation where an enzyme (prenyltransferases) then attaches a hydrophobic molecule (geranylgeranyl

or farnesyl) (See, e.g., Running et al. *Proc Natl Acad Sci USA* 101: 7815-7820 (2004); Maurer-Stroh et al. *Genome Biology* 4:212 (2003)). This hydrophobic addition guides and associates the protein to a membrane (in case of the cytosol, the membrane would be the plasmamembrane or the cytosolic site of the nuclear membrane (Polychronidou et al.

5 Molecular Biology of the Cell 21: 3409-3420 (2010)). More specifically, in representative embodiments, a protein prenyltransferase can catalyze the covalent attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid to C-terminal cysteines of selected proteins carrying a CaaX motif where C=cysteine; a=aliphatic amino acid; x=any amino acid. For plants, this motif most often is CVVQ. The addition of prenyl groups facilitates membrane
10 association and protein-protein interactions of the prenylated proteins.

In still other embodiments of the invention, a signal peptide can direct a SOR, a CO₂ transporter or a rubrerythrin reductase to more than one organelle (e.g., dual targeting). Thus, in some embodiments, a signal peptide that can target the polypeptides to more than one organelle is encoded by an amino acid sequence that includes, but is not limited to, the amino
15 acid sequence of **SEQ ID NO:16**.

In some embodiments, the heterologous polynucleotide encoding a SOR from an archaeon species, the heterologous polynucleotide encoding a CO₂ transporter (e.g., aquaporin) and/or the heterologous polynucleotide encoding an archaeon rubrerythrin reductase can be comprised within an expression cassette. As used herein, “expression
20 cassette” means a recombinant nucleic acid molecule comprising at least one nucleotide sequence of interest (e.g., the heterologous polynucleotide encoding SOR, the heterologous polynucleotide encoding a CO₂ transporter and/or the heterologous polynucleotide encoding an archaeon rubrerythrin reductase), wherein said heterologous polynucleotide is operably associated with at least a control sequence (e.g., a promoter). Thus, some embodiments of
25 the invention provide expression cassettes designed to express a polynucleotide encoding an archaeon SOR, a polynucleotide encoding a CO₂ transporter and/or a polynucleotide encoding rubrerythrin reductase. In this manner, for example, a promoter operably associated with a heterologous polynucleotide encoding a SOR from an archaeon species (e.g., **SEQ ID NO:1** or **SEQ ID NO:2**), and/or functional fragment thereof) are provided in expression
30 cassettes for expression in a plant, plant part, plant cell, bacterial cell and/or yeast cell. In other embodiments, a promoter operably associated with a heterologous polynucleotide encoding a CO₂ transporter (e.g., **SEQ ID NO:58**, **SEQ ID NO:59** or **SEQ ID NO:60**), and/or functional fragment thereof) are provided in expression cassettes for expression in a plant, plant part, plant cell, bacterial cell and/or yeast cell. In this manner, for example, a

promoter operably associated with a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species (e.g., **SEQ ID NO:50**), and/or functional fragment thereof) are provided in expression cassettes for expression in a plant, plant part, plant cell, bacterial cell and/or yeast cell.

5 In some embodiments, the heterologous polynucleotide encoding a SOR can be operably linked to the same promoter that is operably linked to the heterologous polynucleotide encoding a CO₂ transporter and/or the heterologous polynucleotide encoding a rubrerythrin reductase. In other embodiments, the heterologous polynucleotide encoding a SOR is operably linked to a different promoter than that which is operably linked to the
10 heterologous polynucleotide encoding a CO₂ transporter and/or the heterologous polynucleotide encoding a rubrerythrin reductase.

An expression cassette comprising a heterologous polynucleotide encoding a SOR and/or a CO₂ transporter may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. An expression cassette may
15 also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

Any promoter useful for initiation of transcription in a cell of a plant, yeast or bacteria can be used in the expression cassettes of the present invention. A “promoter,” as used herein, is a nucleotide sequence that controls or regulates the transcription of a nucleotide
20 sequence (*i.e.*, a coding sequence) that is operably associated with the promoter. The coding sequence may encode a polypeptide and/or a functional RNA. Typically, a “promoter” refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5', or upstream, relative to the start of the coding region of the corresponding coding sequence. The promoter region may
25 comprise other elements that act as regulators of gene expression. These include a TATA box consensus sequence, and often a CAAT box consensus sequence (Breathnach and Chambon, (1981) *Annu. Rev. Biochem.* 50:349). In plants, the CAAT box may be substituted by the AGGA box (Messing *et al.*, (1983) in *Genetic Engineering of Plants*, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227).

30 Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and/or tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, *i.e.*, “chimeric genes” or “chimeric polynucleotides.” A promoter can be identified in and isolated from the

organism to be transformed and then inserted into the nucleic acid construct to be used in transformation of the organism.

The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. Thus, for example, expression of the heterologous polynucleotide encoding an archaeon SOR can be in any plant, plant part, (e.g., in leaves, in stalks or stems, in ears, in inflorescences (e.g. spikes, panicles, cobs, etc.), in roots, seeds and/or seedlings, and the like), plant cells (including algae cells), yeast cells, or bacterial cells. For example, in the case of a multicellular organism such as a plant where expression in a specific tissue or organ is desired, a tissue-specific or tissue preferred promoter can be used (e.g., a root specific/preferred promoter). In contrast, where expression in response to a stimulus is desired a promoter inducible by stimuli or chemicals can be used. Where continuous expression at a relatively constant level is desired throughout the cells or tissues of an organism a constitutive promoter can be chosen.

Thus, promoters useful with the invention include, but are not limited to, those that drive expression of a nucleotide sequence constitutively, those that drive expression when induced, and those that drive expression in a tissue- or developmentally-specific manner. These various types of promoters are known in the art. Promoters can be identified in and isolated from the plant, yeast, or bacteria to be transformed and then inserted into the expression cassette to be used in transformation of the plant, yeast, or bacteria.

Non-limiting examples of a promoter include the promoter of the RubisCo small subunit gene 1 (PrbcS1), the promoter of the actin gene (Pactin), the promoter of the nitrate reductase gene (Pnr) and the promoter of duplicated carbonic anhydrase gene 1 (Pdca1) (*See, Walker et al. Plant Cell Rep. 23:727-735 (2005); Li et al. Gene 403:132-142 (2007); Li et al. Mol Biol. Rep. 37:1143-1154 (2010)*). PrbcS1 and Pactin are constitutive promoters and Pnr and Pdca1 are inducible promoters. Pnr is induced by nitrate and repressed by ammonium (Li et al. *Gene 403:132-142 (2007)*) and Pdca1 is induced by salt (Li et al. *Mol Biol. Rep. 37:1143-1154 (2010)*).

Examples of constitutive promoters useful for plants include, but are not limited to, cestrum virus promoter (cmp) (U.S. Patent No. 7,166,770), the rice actin 1 promoter (Wang *et al. (1992) Mol. Cell. Biol. 12:3399-3406*; as well as US Patent No. 5,641,876), CaMV 35S promoter (Odell *et al. (1985) Nature 313:810-812*), CaMV 19S promoter (Lawton *et al. (1987) Plant Mol. Biol. 9:315-324*), nos promoter (Ebert *et al. (1987) Proc. Natl. Acad. Sci USA 84:5745-5749*), Adh promoter (Walker *et al. (1987) Proc. Natl. Acad. Sci. USA*

84:6624-6629), sucrose synthase promoter (Yang & Russell (1990) *Proc. Natl. Acad. Sci. USA* 87:4144-4148), and the ubiquitin promoter. The constitutive promoter derived from ubiquitin accumulates in many cell types. Ubiquitin promoters have been cloned from several plant species for use in transgenic plants, for example, sunflower (Binet *et al.*, 1991. *Plant Science* 79: 87-94), maize (Christensen *et al.*, 1989. *Plant Molec. Biol.* 12: 619-632), and arabidopsis (Norris *et al.* 1993. *Plant Molec. Biol.* 21:895-906). The maize ubiquitin promoter (*UbiP*) has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926. The ubiquitin promoter is suitable for the expression of the nucleotide sequences of the invention in transgenic plants, especially monocotyledons. Further, the promoter expression cassettes described by McElroy *et al.* (*Mol. Gen. Genet.* 231: 150-160 (1991)) can be easily modified for the expression of the nucleotide sequences of the invention and are particularly suitable for use in monocotyledonous hosts.

In some embodiments, tissue specific/tissue preferred promoters can be used for expression of a heterologous polynucleotide in a plant cell. Tissue specific or preferred expression patterns include, but are not limited to, green tissue specific or preferred, root specific or preferred, stem specific or preferred, and flower specific or preferred. Promoters suitable for expression in green tissue include many that regulate genes involved in photosynthesis and many of these have been cloned from both monocotyledons and dicotyledons. In one embodiment, a promoter useful with the invention is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, *Plant Molec. Biol.* 12:579-589 (1989)). Non-limiting examples of tissue-specific promoters include those associated with genes encoding the seed storage proteins (such as β -conglycinin, cruciferin, napin and phaseolin), zein or oil body proteins (such as oleosin), or proteins involved in fatty acid biosynthesis (including acyl carrier protein, stearyl-ACP desaturase and fatty acid desaturases (fad 2-1)), and other nucleic acids expressed during embryo development (such as Bce4, *see, e.g.*, Kridl *et al.* (1991) *Seed Sci. Res.* 1:209-219; as well as EP Patent No. 255378). Tissue-specific or tissue-preferential promoters useful for the expression of the nucleotide sequences of the invention in plants, particularly maize, include but are not limited to those that direct expression in root, pith, leaf or pollen. Such promoters are disclosed, for example, in WO 93/07278, herein incorporated by reference in its entirety. Other non-limiting examples of tissue specific or tissue preferred promoters useful with the invention the cotton rubisco promoter disclosed in US Patent 6,040,504; the rice sucrose synthase promoter disclosed in US Patent 5,604,121; the root specific promoter described by de

Framond (FEBS 290:103-106 (1991); EP 0 452 269 to Ciba- Geigy); the stem specific promoter described in U.S. Patent 5,625,136 (to Ciba-Geigy) and which drives expression of the maize trpA gene; and the cestrum yellow leaf curling virus promoter disclosed in WO 01/73087.

5 Additional examples of plant tissue-specific/tissue preferred promoters include, but are not limited to, the root hair-specific cis-elements (RHEs) (Kim et al. *The Plant Cell* 18:2958-2970 (2006)), the root-specific promoters *RCc3* (Jeong et al. *Plant Physiol.* 153:185-197 (2010)) and RB7 (U.S. Patent No. 5459252), the lectin promoter (Lindstrom et al. (1990) *Der. Genet.* 11:160-167; and Vodkin (1983) *Prog. Clin. Biol. Res.* 138:87-98), corn alcohol dehydrogenase 1 promoter (Dennis et al. (1984) *Nucleic Acids Res.* 12:3983-4000), S-adenosyl-L-methionine synthetase (SAMS) (Vander Mijnsbrugge et al. (1996) *Plant and Cell Physiology*, 37(8):1108-1115), corn light harvesting complex promoter (Bansal et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3654-3658), corn heat shock protein promoter (O'Dell et al. (1985) *EMBO J.* 5:451-458; and Rochester et al. (1986) *EMBO J.* 5:451-458), pea small subunit RuBP carboxylase promoter (Cashmore, "Nuclear genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase" pp. 29-39 In: *Genetic Engineering of Plants* (Hollaender ed., Plenum Press 1983; and Poulsen et al. (1986) *Mol. Gen. Genet.* 205:193-200), Ti plasmid mannopine synthase promoter (Langridge et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3219-3223), Ti plasmid nopaline synthase promoter (Langridge et al. (1989),
20 *supra*), petunia chalcone isomerase promoter (van Tunen et al. (1988) *EMBO J.* 7:1257-1263), bean glycine rich protein 1 promoter (Keller et al. (1989) *Genes Dev.* 3:1639-1646), truncated CaMV 35S promoter (O'Dell et al. (1985) *Nature* 313:810-812), potato patatin promoter (Wenzler et al. (1989) *Plant Mol. Biol.* 13:347-354), root cell promoter (Yamamoto et al. (1990) *Nucleic Acids Res.* 18:7449), maize zein promoter (Kriz et al. (1987) *Mol. Gen. Genet.* 207:90-98; Langridge et al. (1983) *Cell* 34:1015-1022; Reina et al. (1990) *Nucleic Acids Res.* 18:6425; Reina et al. (1990) *Nucleic Acids Res.* 18:7449; and Wandelt et al. (1989) *Nucleic Acids Res.* 17:2354), globulin-1 promoter (Belanger et al. (1991) *Genetics* 129:863-872), α -tubulin cab promoter (Sullivan et al. (1989) *Mol. Gen. Genet.* 215:431-440), PEPCase promoter (Hudspeth & Grula (1989) *Plant Mol. Biol.* 12:579-589), R gene
30 complex-associated promoters (Chandler et al. (1989) *Plant Cell* 1:1175-1183), and chalcone synthase promoters (Franken et al. (1991) *EMBO J.* 10:2605-2612).

Particularly useful for seed-specific expression is the pea vicilin promoter (Czako et al. (1992) *Mol. Gen. Genet.* 235:33-40; as well as the seed-specific promoters disclosed in U.S. Patent No. 5,625,136. Useful promoters for expression in mature leaves are those that

are switched at the onset of senescence, such as the SAG promoter from *Arabidopsis* (Gan *et al.* (1995) *Science* 270:1986-1988).

In addition, promoters functional in chloroplasts can be used. Non-limiting examples of such promoters include the bacteriophage T3 gene 9 5' UTR and other promoters disclosed in U.S. Patent No. 7,579,516. Other promoters useful with the invention include but are not limited to the S-E9 small subunit RuBP carboxylase promoter and the Kunitz trypsin inhibitor gene promoter (Kti3).

In some embodiments of the invention, inducible promoters can be used. Thus, for example, chemical-regulated promoters can be used to modulate the expression of a gene in an organism through the application of an exogenous chemical regulator. Regulation of the expression of nucleotide sequences of the invention via promoters that are chemically regulated enables the polypeptides of the invention to be synthesized only when, for example, a crop of plants are treated with the inducing chemicals. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of a chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression.

Chemical inducible promoters useful with plants are known in the art and include, but are not limited to, the maize *In2-2* promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco *PR-1a* promoter, which is activated by salicylic acid (*e.g.*, the PR1a system), steroid-responsive promoters (*see, e.g.*, the glucocorticoid-inducible promoter in Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88, 10421-10425 and McNellis *et al.* (1998) *Plant J.* 14, 247-257) and tetracycline-inducible and tetracycline-repressible promoters (*see, e.g.*, Gatz *et al.* (1991) *Mol. Gen. Genet.* 227, 229-237, and U.S. Patent Numbers 5,814,618 and 5,789,156, Lac repressor system promoters, copper-inducible system promoters, salicylate-inducible system promoters (*e.g.*, the PR1a system), glucocorticoid-inducible promoters (Aoyama *et al.* (1997) *Plant J.* 11:605-612), and ecdysone-inducible system promoters.

Other non-limiting examples of inducible promoters include ABA- and turgor-inducible promoters, the auxin-binding protein gene promoter (Schwob *et al.* (1993) *Plant J.* 4:423-432), the UDP glucose flavonoid glycosyl-transferase promoter (Ralston *et al.* (1988) *Genetics* 119:185-197), the MPI proteinase inhibitor promoter (Cordero *et al.* (1994) *Plant J.* 6:141-150), and the glyceraldehyde-3-phosphate dehydrogenase promoter (Kohler *et al.* (1995) *Plant Mol. Biol.* 29:1293-1298; Martinez *et al.* (1989) *J. Mol. Biol.* 208:551-565; and

Quigley *et al.* (1989) *J. Mol. Evol.* 29:412-421). Also included are the benzene sulphonamide-inducible (US Patent No. 5,364,780) and alcohol-inducible (Int'l Patent Application Publication Nos. WO 97/06269 and WO 97/06268) systems and glutathione S-transferase promoters. Likewise, one can use any of the inducible promoters described in
 5 Gatz (1996) *Current Opinion Biotechnol.* 7:168-172 and Gatz (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:89-108. Other chemically inducible promoters useful for directing the expression of the nucleotide sequences of this invention in plants are disclosed in US Patent 5,614,395 herein incorporated by reference in its entirety. Chemical induction of gene expression is also detailed in the published application EP 0 332 104 (to Ciba-Geigy)
 10 and U.S. Patent 5,614,395. In some embodiments, a promoter for chemical induction can be the tobacco PR-1a promoter.

In some particular embodiments, promoters useful with algae include, but are not limited to, the promoter of the RubisCo small subunit gene 1 (PrbcS1), the promoter of the actin gene (Pactin), the promoter of the nitrate reductase gene (Pnr) and the promoter of
 15 duplicated carbonic anhydrase gene 1 (Pdca1) (*See*, Walker *et al.* *Plant Cell Rep.* 23:727-735 (2005); Li *et al.* *Gene* 403:132-142 (2007); Li *et al.* *Mol Biol. Rep.* 37:1143-1154 (2010)), the promoter of the σ^{70} -type plastid rRNA gene (Prnr), the promoter of the psbA gene (encoding the photosystem-II reaction center protein D1) (PpsbA), the promoter of the psbD gene (encoding the photosystem-II reaction center protein D2) (PpsbD), the promoter of the psaA
 20 gene (encoding an apoprotein of photosystem I) (PpsaA), the promoter of the ATPase alpha subunit gene (PatpA), and promoter of the RuBisCo large subunit gene (PrbcL), and any combination thereof (*See, e.g.*, De Cosa *et al.* *Nat. Biotechnol.* 19:71-74 (2001); Daniell *et al.* *BMC Biotechnol.* 9:33 (2009); Muto *et al.* *BMC Biotechnol.* 9:26 (2009); Surzycki *et al.* *Biologicals* 37:133-138 (2009)).

25 In some embodiments, promoters useful with bacteria and yeast include, but are not limited to, a constitutive promoter (e.g., *lpp* (lipoprotein gene)) and/or an oxidative stress inducible promoter (e.g., a superoxide dismutase or a catalase promoter).

Thus, in some embodiments, a promoter useful with yeast can include, but is not limited to, a promoter from phosphoglycerate kinase (*PGK*), glyceraldehyde-3-phosphate
 30 dehydrogenase (*GAP*), triose phosphate isomerase (*TPI*), galactose-regulon (*GAL1*, *GAL10*), alcohol dehydrogenase (*ADH1*, *ADH2*), phosphatase (*PHO5*), copper-activated metallothionine (*CUP1*), *MFa1*, *PGK/α2* operator, *TPI/α2* operator, *GAP/GAL*, *PGK/GAL*, *GAP/ADH2*, *GAP/PHO5*, iso-1-cytochrome c/glucocorticoid response element (*CYC/GRE*), phosphoglycerate kinase/angrogen response element (*PGK/ARE*), transcription elongation

factor EF-1 α (*TEF1*), triose phosphate dehydrogenase (*TDH3*), phosphoglycerate kinase 1 (*PGK1*), pyruvate kinase 1 (*PYK1*), and/or hexose transporter (*HXT7*) (See, Romanos et al. *Yeast* 8:423-488 (1992); and Partow et al. *Yeast* 27:955-964 (2010)).

In additional embodiments, a promoter useful with bacteria can include, but is not limited to, L-arabinose inducible (*araBAD*, *P_{BAD}*) promoter, any *lac* promoter, L-rhamnose inducible (*rhaP_{BAD}*) promoter, T7 RNA polymerase promoter, *trc* promoter, *tac* promoter, lambda phage promoter (*p_L*, *p_L-9G-50*), anhydrotetracycline-inducible (*tetA*) promoter, *trp*, *lpp*, *phoA*, *recA*, *proU*, *cst-1*, *cadA*, *nar*, *lpp-lac*, *cspA*, T7-*lac* operator, T3-*lac* operator, T4 gene 32, T5-*lac* operator, *nprM-lac* operator, Vhb, Protein A, corynebacterial-*E. coli* like promoters, *thr*, *hom*, diphtheria toxin promoter, *sig A*, *sig B*, *nusG*, *SoxS*, *kath*, α -amylase (*Pamy*), *Ptms*, P43 (comprised of two overlapping RNA polymerase σ factor recognition sites, σA , σB), *Ptms*, P43, *rplK-rplA*, ferredoxin promoter, and/or xylose promoter. (See, K. Terpe *Appl. Microbiol. Biotechnol.* 72:211-222 (2006); Hannig et al. *Trends in Biotechnology* 16:54-60 (1998); and Srivastava *Protein Expr Purif* 40:221-229 (2005)).

In addition to promoters operably linked to a heterologous polynucleotide encoding an archaeal SOR (e.g., a *P. furiosus* SOR, **SEQ ID NO:1**, **SEQ ID NO:2**), and promoters operably linked to a heterologous polynucleotide encoding a CO₂ transporter (e.g., **SEQ ID NO:58**, **SEQ ID NO:60**, **SEQ ID NO:62**) an expression cassette also can include other regulatory sequences. As used herein, “regulatory sequences” means nucleotide sequences located upstream (5' non-coding sequences), within or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences include, but are not limited to, enhancers, introns, translation leader sequences, translation termination sequences, and polyadenylation signal sequences, as described herein.

Thus, in some embodiments of the present invention, the expression cassettes can include at least one intron. An intron useful with this invention can be an intron identified in and isolated from a plant, yeast, or bacteria to be transformed and then inserted into the expression cassette to be used in transformation of the plant, yeast, or bacteria. As would be understood by those of skill in the art, the introns as used herein comprise the sequences required for self excision and are incorporated into the nucleic acid constructs in frame. An intron can be used either as a spacer to separate multiple protein-coding sequences in one nucleic acid construct, or an intron can be used inside one protein-coding sequence to stabilize the mRNA. If they are used within a protein-coding sequence, they are inserted “in-frame” with the excision sites included.

Non-limiting examples of introns useful with the present invention can be introns from the RuBisCO small subunit (*rbcS*) gene, the RuBisCO large subunit (*rbcL*) gene, the actin gene, the nitrate reductase gene (*nr*), the duplicated carbonic anhydrase gene 1 (*Tdca1*), the *psbA* gene, the *atpA* gene, or any combination thereof.

5 In some embodiments of the invention, an expression cassette can comprise an enhancer sequence. Enhancer sequences can be derived from, for example, any intron from any highly expressed gene. In particular embodiments, an enhancer sequence usable with this invention includes, but is not limited to, the nucleotide sequence of ggagg (e.g., ribosome binding site).

10 An expression cassette also can optionally include a transcriptional and/or translational termination region (*i.e.*, termination region) that is functional in plants, yeast or bacteria. A variety of transcriptional terminators are available for use in expression cassettes and are responsible for the termination of transcription beyond the heterologous polynucleotide of interest and correct mRNA polyadenylation. The termination region may
15 be native to the transcriptional initiation region, may be native to the operably linked nucleotide sequence of interest, may be native to the host cell, or may be derived from another source (*i.e.*, foreign or heterologous to the promoter, the nucleotide sequence of interest, the host cell, or any combination thereof). Non-limiting examples of transcriptional terminators useful for plants can be a CAMV 35S terminator, a *tml* terminator, a nopaline synthase terminator and/or a pea *rbcS* E9 terminator, a RubisCo small subunit gene 1
20 (*TrbcS1*) terminator, an actin gene (*Tactin*) terminator, a nitrate reductase gene (*Tnr*) terminator, and/or a duplicated carbonic anhydrase gene 1 (*Tdca1*) terminator.

Further non-limiting examples of terminators useful with this invention for expression of SOR or other heterologous polynucleotides in algae include a terminator of the *psbA* gene
25 (*TpsbA*), a terminator of the *psaA* gene (encoding an apoprotein of photosystem I) (*TpsaA*), a terminator of the *psbD* gene (*TpsbD*), a RuBisCo large subunit terminator (*TrbcL*), a terminator of the σ^{70} -type plastid rRNA gene (*Trrn*), and/or a terminator of the ATPase alpha subunit gene (*TatpA*).

Non-limiting examples of terminators for use with bacteria can be from *trp*, *hom-trpB*,
30 *lysA*, *thrB*, and/or *sodA*.

An expression cassette of the invention also can include a nucleotide sequence for a selectable marker, which can be used to select a transformed plant, plant part, plant cell, yeast cell or bacteria cell. As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to a plant, plant part, plant cell, yeast cell or

bacterial cell expressing the marker and thus allows such a transformed plant, plant part, plant cell, yeast cell or bacterial cell to be distinguished from that which does not have the marker. Such a nucleotide sequence may encode either a selectable or screenable marker, depending on whether the marker confers a trait that can be selected for by chemical means, such as by using a selective agent (*e.g.*, an antibiotic, herbicide, or the like), or whether the marker is simply a trait that one can identify through observation or testing, such as by screening (*e.g.*, the R-locus trait). Of course, many examples of suitable selectable markers are known in the art and can be used in the expression cassettes described herein.

Examples of selectable markers include, but are not limited to, a nucleotide sequence encoding *aadA* (*i.e.*, spectinomycin and streptomycin resistance), a nucleotide sequence encoding *neo* (*i.e.*, kanamycin resistance), a nucleotide sequence encoding *aphA6* (*i.e.*, kanamycin resistance), a nucleotide sequence encoding *nptII* (*i.e.*, kanamycin resistance), a nucleotide sequence encoding *bar* (*i.e.*, phosphinothricin resistance), a nucleotide sequence encoding *cat* (*i.e.*, chloramphenicol resistance), a nucleotide sequence encoding *badh* (*i.e.*, betaine aldehyde resistance), a nucleotide sequence encoding *egfp*, (*i.e.*, enhanced green fluorescence protein), a nucleotide sequence encoding *gfp* (*i.e.*, green fluorescent protein), a nucleotide sequence encoding mCherry (mCherry or red fluorescent protein), a nucleotide sequence encoding *luc* (*i.e.*, luciferase), a nucleotide sequence encoding *ble* (bleomycin resistance), a nucleotide sequence encoding *ereA* (erythromycin resistance), and any combination thereof.

Further examples of selectable markers useful with the invention include, but are not limited to, a nucleotide sequence encoding an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which confers resistance to glyphosate (Hinchee *et al.* (1988) *Biotech.* 6:915-922); a nucleotide sequence encoding a nitrilase such as *bxn* from *Klebsiella ozaenae* that confers resistance to bromoxynil (Stalker *et al.* (1988) *Science* 242:419-423); a nucleotide sequence encoding an altered acetolactate synthase (ALS) that confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (EP Patent Application No. 154204); a nucleotide sequence encoding a methotrexate-resistant dihydrofolate reductase (DHFR) (Thillet *et al.* (1988) *J. Biol. Chem.* 263:12500-12508); a nucleotide sequence encoding a dalapon dehalogenase that confers resistance to dalapon; a nucleotide sequence encoding a mannose-6-phosphate isomerase (also referred to as phosphomannose isomerase (PMI)) that confers an ability to metabolize mannose (US Patent Nos. 5,767,378 and 5,994,629); a nucleotide sequence encoding an altered anthranilate synthase that confers

resistance to 5-methyl tryptophan; and/or a nucleotide sequence encoding *hph* that confers resistance to hygromycin.

Additional selectable markers include, but are not limited to, a nucleotide sequence encoding β -glucuronidase or *uidA* (GUS) that encodes an enzyme for which various chromogenic substrates are known; an R-locus nucleotide sequence that encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, "Molecular cloning of the maize R-nj allele by transposon-tagging with Ac" 263-282 In: *Chromosome Structure and Function: Impact of New Concepts*, 18th Stadler Genetics Symposium (Gustafson & Appels eds., Plenum Press 1988)); a nucleotide sequence encoding β -lactamase, an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin) (Sutcliffe (1978) *Proc. Natl. Acad. Sci. USA* 75:3737-3741); a nucleotide sequence encoding *xylE* that encodes a catechol dioxygenase (Zukowsky *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1101-1105); a nucleotide sequence encoding tyrosinase, an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone, which in turn condenses to form melanin (Katz *et al.* (1983) *J. Gen. Microbiol.* 129:2703-2714); a nucleotide sequence encoding β -galactosidase, an enzyme for which there are chromogenic substrates; a nucleotide sequence encoding luciferase (*lux*) that allows for bioluminescence detection (Ow *et al.* (1986) *Science* 234:856-859); a nucleotide sequence encoding *Bla* that confers ampicillin resistance; or a nucleotide sequence encoding aequorin which may be employed in calcium-sensitive bioluminescence detection (Prasher *et al.* (1985) *Biochem. Biophys. Res. Comm.* 126:1259-1268), and/or any combination thereof. In some embodiments, an expression cassette can further encode green fluorescent protein (GFP). One of skill in the art is capable of choosing a suitable selectable marker for use in an expression cassette of this invention.

An expression cassette comprising a heterologous polynucleotide encoding a SOR and an expression cassette comprising a heterologous polynucleotide encoding a CO₂ transporter and a SOR also can include polynucleotides that encode other desired traits. Such desired traits can be polynucleotides which confer high light tolerance, increased drought tolerance, increased flooding tolerance, increased tolerance to soil contaminants, increased CO₂ uptake, increased CO₂ assimilation, modification of carbon flux, increased yield, modified fatty acid composition of the lipids, increased oil production in seed, increased and modified starch production in seeds, increased and modified protein production in seeds, modified tolerance to herbicides and pesticides, production of terpenes, increased seed number, increased biomass of the roots, increased and/or modified biomass of the stem

(trees), increased and/or modified biomass of the leaves, reduced photorespiration, and/or other desirable traits for agriculture or biotechnology.

Such polynucleotides can be stacked with any combination of nucleotide sequences to create plants, plant parts, plant cells, yeast cells or bacterial cells having the desired

5 phenotype. Stacked combinations can be created by any method including, but not limited to, any conventional methodology (e.g., cross breeding for plants), or by genetic transformation.

If stacked by genetic transformation, nucleotide sequences encoding additional desired traits can be combined at any time and in any order. For example, a transgenic plant comprising

10 one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The additional nucleotide sequences can be introduced simultaneously in a co-transformation protocol with a nucleotide sequence, nucleic acid molecule, nucleic acid

construct, and/or other composition of the invention, provided by any combination of expression cassettes. For example, if two nucleotide sequences will be introduced, they can

be incorporated in separate cassettes (*trans*) or can be incorporated on the same cassette (*cis*).

15 Expression of the nucleotide sequences can be driven by the same promoter or by different promoters. It is further recognized that nucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. *See, e.g.*, Int'l Patent

Application Publication Nos. WO 99/25821; WO 99/25854; WO 99/25840; WO 99/25855 and WO 99/25853.

20 By “operably linked” or “operably associated,” it is meant that the indicated elements are functionally related to each other, and are also generally physically related. Thus, the term “operably linked” or “operably associated” as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Therefore, a first nucleotide

sequence that is operably linked to a second nucleotide sequence means a situation when the

25 first nucleotide sequence is placed in a functional relationship with the second nucleotide sequence. For instance, a promoter is operably associated with a nucleotide sequence if the promoter effects the transcription or expression of said nucleotide sequence. Those skilled in

the art will appreciate that the control sequences (e.g., promoter) need not be contiguous with the nucleotide sequence to which it is operably associated, as long as the control sequences

30 function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a nucleotide sequence, and the promoter can still be considered “operably linked” to the nucleotide sequence.

Methods for detecting and quantifying ROS or oxidized cell components are well known in the art and include, but are not limited to: the nitroblue tetrazolium assay (Fryer et

al. *J Exp Bot* 53: 1249–1254 (2002); Fryer et al. *Plant J* 33: 691–705 (2003)) and acridan lumigen PS-3 assay (Uy et al. *Journal of Biomolecular Techniques* 22:95–107 (2011) for detection of superoxide; the ferrous ammonium sulfate/xylene orange (FOX) method (Wolff, *Methods Enzymol* 233: 182–189 (1994); Im et al. *Plant Physiol* 151:893–904(2009)) for
 5 detection of peroxide; the 3,3-Diaminobenzidine (DAB) assay (Thordal-Christensen et al. *Plant J* 11(6): 1187-1194 (1997); the thiobarbituric acid assay (TBA) (Draper and Hadley, *Methods Enzymol* 186:421–431 (1990); Hodges et al. *Planta* 207: 604–611 (1999)) and the mass spectrometric determination of peroxidized lipids (Deighton et al. *Free Radic Res* 27: 255–265 (1997)) for detection of lipid peroxidation; the assay for 8-hydroxy-2'-
 10 deoxyguanosine in DNA (Bialkowski and Olinski, *Acta Biochim Pol* 46: 43–49 (1999)) for the detection of nucleic acid oxidation; and the reaction of oxidized protein with 2,4-dinitrophenylhydrazine (DPNH) (Levine et al. *Methods Enzymol* 233:346–357 (1994)) for detection of protein oxidation.

Methods for measuring photorespiration are known in the art. Thus, photorespiration
 15 can be indirectly measured by changes in the CO₂-saturation curve using fluorescence and gas exchange measurements (e.g., LiCOR) or via ¹⁸O₂ incorporation. Alternatively, determining the ratio of serine to glycine in actively photosynthesizing leaves can be used to measure photorespiration. Other ways that changes in photorespiration can be shown include comparing biomass productivity or photosynthesis under different CO₂:O₂ environments.
 20 See, e.g., Hideg et al. *Plant and Cell Physiology* 49: 1879-1886 (2008); and Berry et al. *Plant Physiol* 62:954-967 (1978).

Photosynthetic efficiency is the fraction of light energy converted into chemical energy during photosynthesis. Saturating pulse fluorescence measurements can be used to measure photosynthetic efficiency. CO₂ and O₂ exchange methods can also be used. A
 25 number of plant and algae studies have been done, which demonstrate that photosynthetic efficiency decreases when plants are exposed to ROS (Ganesh et al. *Biotechnol Bioeng* 96(6):1191-8 (2007); Zhang and Xing. *Plant Cell Physiology* 49(7):1092-1111(2008).

Reactive oxygen species (ROS) are generated in the cells of aerobic organisms during normal metabolic processes and have been identified to have an important role in cell
 30 signaling and homeostasis. However, high levels of ROS can be detrimental to an organism's cell structure and metabolism often resulting in cell death (i.e., oxidative stress). Most organisms have endogenous mechanisms for protecting them from potential damage by ROS, including enzymes such as superoxide dismutase, catalase and peroxide, and small antioxidant molecules. However, under conditions of abiotic stress, the levels of ROS can

rise significantly making the endogenous protective mechanisms insufficient. By stably introducing a heterologous polynucleotide encoding SOR from an archaeon species into the cells of plants, bacteria and yeast as described herein, said plants, yeasts and bacteria stably expressing the SOR have increased tolerance to the environmental stresses that induce ROS production.

“Abiotic stress” or “environmental stress” as used herein means any outside, nonliving, physical or chemical factors or conditions that induce ROS production. Thus, in some embodiments of the invention, an abiotic or environmental stress can include, but is not limited to, high heat, high light, ultraviolet radiation, high salt, drought, ozone, heavy metals, pesticides, herbicides, toxins, and/or anoxia (i.e., root flooding).

Parameters for the abiotic stress factors are species specific and even variety specific and therefore vary widely according to the species/variety exposed to the abiotic stress. Thus, for example, while one species may be severely impacted by a high temperature of 23°C, another species may not be impacted until at least 30°C, and the like. Temperatures above 30°C result in, for example, dramatic reductions in the yields of many plant crops including algae. This is due to reductions in photosynthesis that begin at approximately 20-25°C, and the increased carbohydrate demands of crops growing at higher temperatures. The critical temperatures are not absolute, but vary depending upon such factors as the acclimatization of the organism to prevailing environmental conditions. In addition, because organisms are often exposed to multiple abiotic stresses at one time, the interaction between the stresses affects the response. For example, damage to a plant from excess light occurs at lower light intensities as temperatures increase beyond the photosynthetic optimum. Water stressed plants are less able to cool overheated tissues due to reduced transpiration, further exacerbating the impact of excess (high) heat and/or excess (high) light intensity. Thus, the particular parameters for high/low temperature, light intensity, drought and the like, which can negatively impact an organism will vary with species, variety, degree of acclimatization and the exposure to a combination of environmental conditions.

Any plant (or groupings of plants, for example, into a genus or higher order classification) can be employed in practicing the present invention including an angiosperm, a gymnosperm, a monocot, a dicot, a C3, C4, CAM plant, a microalgae, and/or a macroalgae.

The term “plant part,” as used herein, includes but is not limited to reproductive tissues (e.g., petals, sepals, stamens, pistils, receptacles, anthers, pollen, flowers, fruits, flower bud, ovules, seeds, embryos, nuts, kernels, ears, cobs and husks); vegetative tissues (e.g., petioles, stems, roots, root hairs, root tips, pith, coleoptiles, stalks, shoots, branches,

bark, apical meristem, axillary bud, cotyledon, hypocotyls, trichomes, and leaves); vascular tissues (e.g., phloem and xylem); specialized cells such as epidermal cells, parenchyma cells, chlorenchyma cells, schlerenchyma cells, stomates, guard cells, cuticle, mesophyll cells; callus tissue; and cuttings. The term “plant part” also includes plant cells, including plant
 5 cells that are intact in plants and/or parts of plants, plant protoplasts, plant tissues, plant organs, plant cell tissue cultures, plant calli, plant clumps, and the like. As used herein, “shoot” refers to the above ground parts including the leaves and stems. As used herein, the term “tissue culture” encompasses cultures of tissue, cells, protoplasts and callus.

As used herein, “plant cell” refers to a structural and physiological unit of the plant,
 10 which typically comprise a cell wall but also includes protoplasts. A plant cell of the present invention can be in the form of an isolated single cell or can be a cultured cell or can be a part of a higher-organized unit such as, for example, a plant tissue (including callus) or a plant organ. In some embodiments, a plant cell can be an algal cell.

In some embodiments of this invention, a plant, plant part or plant cell can be from a
 15 genus including, but not limited to, the genus of *Camelina*, *Glycine*, *Sorghum*, *Brassica*, *Allium*, *Armoracia*, *Poa*, *Agrostis*, *Lolium*, *Festuca*, *Calamagrostis*, *Deschampsia*, *Spinacia*, *Beta*, *Pisum*, *Chenopodium*, *Helianthus*, *Pastinaca*, *Daucus*, *Petroselinum*, *Populus*, *Prunus*, *Castanea*, *Eucalyptus*, *Acer*, *Quercus*, *Salix*, *Juglans*, *Picea*, *Pinus*, *Abies*, *Lemna*, *Wolffia*, *Spirodela*, *Oryza*, *Zea* or *Gossypium*.

In other embodiments, a plant, plant part or plant cell can be from a species including,
 20 but not limited to, the species of *Camelina alyssum* (Mill.) Thell., *Camelina microcarpa* Andrzej. ex DC., *Camelina rumelica* Velen., *Camelina sativa* (L.) Crantz, *Sorghum bicolor* (e.g., *Sorghum bicolor* L. Moench), *Gossypium hirsutum*, *Glycine max*, *Zea mays*

Brassica oleracea, *Brassica rapa*, *Brassica napus*, *Raphanus sativus*, *Armoracia*
 25 *rusticana*, *Allium sativum*, *Allium cepa*, *Populus grandidentata*, *Populus tremula*, *Populus tremuloides*, *Prunus serotina*, *Prunus pensylvanica*, *Castanea dentata*, *Populus balsamifer*, *Populus deltoides*, *Acer saccharum*, *Acer nigrum*, *Acer negundo*, *Acer rubrum*, *Acer saccharinum*, *Acer pseudoplatanus* or *Oryza sativa*. In additional embodiments, the plant, plant part or plant cell can be, but is not limited to, a plant of, or a plant part, or plant cell
 30 from wheat, barley, oats, turfgrass (bluegrass, bentgrass, ryegrass, fescue), feather reed grass, tufted hair grass, spinach, beets, chard, quinoa, sugar beets, lettuce, sunflower (*Helianthus annuus*), peas (*Pisum sativum*), parsnips (*Pastinaca sativa*), carrots (*Daucus carota*), parsley (*Petroselinum crispum*), duckweed, pine, spruce, fir, eucalyptus, oak, walnut, or willow. In particular embodiments, the plant, plant part and/or plant cell can be from *Camelina sativa*.

In other particular embodiments, the plant, plant part and/or plant cell is not from *Arabidopsis thaliana*. In some representative embodiments, the plant, plant part, and/or plant cell is camelina, wheat, rice, corn, rape, canola, soybean, sorghum, or cotton.

In further embodiments, a plant and/or plant cell can be an algae or algae cell from a class including, but not limited to, the class of Bacillariophyceae (diatoms), Haptophyceae, Phaeophyceae (brown algae), Rhodophyceae (red algae) or Glaucophyceae (red algae). In still other embodiments, a plant and/or plant cell can be an algae or algae cell from a genus including, but not limited to, the genus of *Achnanthes*, *Actinella*, *Nitzschia*, *Nupela*, *Geissleria*, *Gomphonema*, *Planothidium*, *Halamphora*, *Psammodium*, *Navicula*, *Eunotia*, *Stauroneis*, *Chlamydomonas*, *Dunaliella*, *Nannochloris*, *Nannochloropsis*, *Scenedesmus*, *Chlorella*, *Cyclotella*, *Amphora*, *Thalassiosira*, *Phaeodactylum*, *Chrysochromulina*, *Prymnesium*, *Thalassiosira*, *Phaeodactylum*, *Glaucocystis*, *Cyanophora*, *Galdieria*, or *Porphyridium*. Additional nonlimiting examples of genera and species of diatoms useful with this invention are provided by the US Geological Survey/Institute of Arctic and Alpine Research at westerndiatoms.colorado.edu/species.

Any bacterium can be employed in practicing the present invention. In particular embodiments, a bacterial cell can be from a phylum that includes, but not limited to, the phylum of Cyanobacteria or can be from a genus including, but not limited to, the genus of *Bacillus*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pseudomonas*, *Corynebacterium*, *Escherichia* or *Clostridium*. In some embodiments, a bacterial cell can be *Escherichia coli*.

Further, any yeast in which heterologous expression of a SOR is useful can be used with the methods of this invention. In some representative embodiments, a yeast cell can be from a genus including, but not limited to, the genus of *Saccharomyces*, *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Candida*, *Zygosaccharomyces* or *Hanseniaspora*. In other embodiments, a yeast cell can be from a species including, but not limited to, the species of *Saccharomyces cerevisiae*, *S. uvarum* (*carlsbergensis*), *S. diastaticus*, *Saccharomyces ludwigii*, *Kluyveromyces marxianus*, *Pichia pastoris*, *Candida stellata*, *C. pulcherrima*, *Zygosaccharomyces fermentati*, or *Hanseniaspora uvarum*.

As described herein, the present invention provides methods for increasing disease resistance (e.g., decrease disease symptoms and/or decreased pathogen growth and reproduction) in a plant, plant cell, or plant part, comprising: introducing into a plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to

a control (e.g., a plant, plant part or plant cell that does not comprise said heterologous polynucleotide encoding a superoxide reductase from an archaeon species).

In some embodiments, the diseases for which an increase in resistance (or a decrease in disease symptoms/decreased pathogen growth and/or reproduction) can be observed

include, but are not limited to, fungal diseases, bacterial diseases, and/or viral disease. Non-limiting examples of diseases include powdery mildew (*Erysiphe* spp.), damping off (*Rhizoctonia solani*), leaf blotch (*Mycosphaerella* spp.), rust (*Puccinia* spp.), leaf mold (*Cladosporium* spp.), soft rot (*Rhizopus* spp.), wilt (*Fusarium* spp.), coffee rust (*Haemelia vastatrix*) and/or *Pseudomonas* spp.

In some embodiments, the plant (and plant part or plant cell therefrom) and the disease for which increased resistance can be observed can include, but is not limited to, *Camelina sativa* and *Erysiphe* spp.; coffee rust and coffee; tomato and potato and potato blight; wheat and leaf rust; tomato and leaf mold; sweet potato and soft rot; corn, sorghum and soybean and charcoal rot; onion and white rot; cucurbit and downy mildew; wheat and black stem rust; and the like.

In representative embodiments, the plant (and plant part or plant cell therefrom) and the disease for which increased resistance can be observed can include, but is not limited to, those provided in **Table 3**.

Table 3. Exemplary diseases

Disease	Pathogen	Host
Powdery mildew	<i>Blumeria graminis</i>	Kentucky blue grass, fine fescue, perennial rye grass
Brown patch	<i>Rhizoctonia solani</i>	Tall fescue
Gray leaf spot	<i>Magnaporthe grisea</i>	Tall fescue
Leaf Spot	<i>Bipolaris</i> spp. or <i>Drechslera</i> spp	Bluegrasses, bermudagrass, fescues or perennial ryegrass
Gray Leaf Spot	(<i>Pyricularia grisea</i>)	Centipedegrass, Perennial ryegrass, St. Augustinegrass or tall fescue
Rust	<i>Puccinia</i> spp	Bermudagrass, bluegrasses, fescues, ryegrasses or zoysiagrass
Southern leaf blight	<i>Cochliobolus heterostrophus</i>	Maize
Blight	<i>Cercospora</i> spp	Tobacco, sugarbeet, Maize,
Blast	<i>Magnaporthe oryzae</i>	Rice
Wilt	<i>Fusarium oxysporum</i>	Tomato and other crops

Any nucleotide sequence to be transformed into a plant, plant part, plant cell, yeast cell or bacterial cell can be modified for codon usage bias using species specific codon usage tables. The codon usage tables are generated based on a sequence analysis of the most highly expressed genes for the species of interest. When the nucleotide sequences are to be

expressed in the nucleus, the codon usage tables are generated based on a sequence analysis of highly expressed nuclear genes for the species of interest. The modifications for the nucleotide sequences for selection are determined by comparing the species specific codon usage table with the codons present in the native nucleotide sequences. In those

5 embodiments in which each of codons in native nucleotide sequence for selection are sufficiently used, then no modifications are needed (e.g., a frequency of more than 30% for a codon used for a specific amino acid in that species would indicate no need for modification). In other embodiments, wherein up to 3 nucleotides have to be modified in the nucleotide sequence, site-directed mutagenesis can be used according to methods known in the art
10 (Zheng et al. *Nucleic Acids Res.* 32:e115 (2004); Dammai, *Meth. Mol. Biol* 634:111-126 (2010); Davis and Vierstra. *Plant Mol. Biol.* 36(4): 521-528 (1998)). In still other embodiments, wherein more than three nucleotide changes are necessary, a synthetic nucleotide sequence can be generated using the same codon usage as the highly expressed genes that were used to develop the codon usage table.

15 The term “transformation” as used herein refers to the introduction of a heterologous polynucleotide into a cell. Transformation of a plant, plant part, plant cell, yeast cell and/or bacterial cell may be stable or transient.

“Transient transformation” in the context of a polynucleotide means that a polynucleotide is introduced into the cell and does not integrate into the genome of the cell.

20 By “stably introducing” or “stably introduced” in the context of a polynucleotide introduced into a cell it is intended that the introduced polynucleotide is stably incorporated into the genome of the cell, and thus the cell is stably transformed with the polynucleotide.

“Stable transformation” or “stably transformed” as used herein means that a nucleic acid molecule is introduced into a cell and integrates into the genome of the cell. As such,
25 the integrated nucleic acid molecule is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations. “Genome” as used herein also includes the nuclear and the plastid genome, and therefore includes integration of the nucleic acid into, for example, the chloroplast genome. Stable transformation as used herein can also refer to a transgene that is maintained extrachromasomally, for example, as a
30 minichromosome.

Transient transformation may be detected by, for example, an enzyme-linked immunosorbent assay (ELISA) or Western blot, which can detect the presence of a peptide or polypeptide encoded by one or more transgene introduced into an organism. Stable transformation of a cell can be detected by, for example, a Southern blot hybridization assay

of genomic DNA of the cell with nucleic acid sequences which specifically hybridize with a nucleotide sequence of a transgene introduced into an organism (e.g., a plant). Stable transformation of a cell can be detected by, for example, a Northern blot hybridization assay of RNA of the cell with nucleic acid sequences which specifically hybridize with a nucleotide sequence of a transgene introduced into a plant or other organism. Stable transformation of a cell can also be detected by, e.g., a polymerase chain reaction (PCR) or other amplification reactions as are well known in the art, employing specific primer sequences that hybridize with target sequence(s) of a transgene, resulting in amplification of the transgene sequence, which can be detected according to standard methods. Transformation can also be detected by direct sequencing and/or hybridization protocols that are well known in the art.

A heterologous polynucleotide encoding a SOR from an archaeon species as described herein and/or fragments thereof, and/or any combination thereof, can be introduced into the cell of a plant, yeast or bacteria by any method known to those of skill in the art. In some embodiments of the invention, transformation of a cell comprises nuclear transformation. In other embodiments, transformation of a cell comprises plastid transformation (e.g., chloroplast transformation).

Procedures for transforming plants, yeast and bacteria are well known and routine in the art and are described throughout the literature. Non-limiting examples of transformation methods include transformation via bacterial-mediated nucleic acid delivery (e.g., via *Agrobacteria*), viral-mediated nucleic acid delivery, silicon carbide or nucleic acid whisker-mediated nucleic acid delivery, liposome mediated nucleic acid delivery, microinjection, microparticle bombardment, calcium-phosphate-mediated transformation, cyclodextrin-mediated transformation, electroporation, nanoparticle-mediated transformation, sonication, infiltration, PEG-mediated nucleic acid uptake, as well as any other electrical, chemical, physical (mechanical) and/or biological mechanism that results in the introduction of nucleic acid into the plant cell, including any combination thereof. General guides to various plant transformation methods known in the art include Miki *et al.* ("Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B. R. and Thompson, J. E., Eds. (CRC Press, Inc., Boca Raton, 1993), pages 67-88) and Rakowoczy-Trojanowska (*Cell. Mol. Biol. Lett.* 7:849-858 (2002)). General guides to the transformation of yeast include Guthrie and Fink (1991) (Guide to yeast genetics and molecular biology. In *Methods in Enzymology*, (Academic Press, San Diego) 194:1-932) and guides to methods related to the transformation of bacteria include Aune and Aachmann (*Appl. Microbiol Biotechnol* 85:1301-1313 (2010)).

A polynucleotide therefore can be introduced into a plant, plant part, plant cell, yeast cell and/or bacterial cell in any number of ways that are well known in the art. The methods of the invention do not depend on a particular method for introducing one or more nucleotide sequences into a plant, only that they gain access to the interior of the cell. Where more than
5 polynucleotide is to be introduced, they can be assembled as part of a single nucleic acid construct, or as separate nucleic acid constructs, and can be located on the same or different nucleic acid constructs. Accordingly, the polynucleotide can be introduced into the cell of interest in a single transformation event, or in separate transformation events, or, alternatively, a polynucleotide can be incorporated into a plant, bacteria or yeast, as part of a
10 breeding protocol.

In some embodiments, when a plant part or plant cell is stably transformed, it can then be used to regenerate a stably transformed plant comprising the heterologous polynucleotide encoding a SOR from an archaeon species in its genome. Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri
15 plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently root. Alternatively, somatic embryo formation can be induced in the callus tissue. These somatic embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and plant hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and
20 proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

The regenerated plants are transferred to standard soil conditions and cultivated in a conventional manner. The plants are grown and harvested using conventional procedures.

25 The particular conditions for transformation, selection and regeneration of a plant can be optimized by those of skill in the art. Factors that affect the efficiency of transformation include the species of plant, the target tissue or cell, composition of the culture media, selectable marker genes, kinds of vectors, and light/dark conditions. Therefore, these and other factors may be varied to determine an optimal transformation protocol for any
30 particular plant species. It is recognized that not every species will react in the same manner to the transformation conditions and may require a slightly different modification of the protocols disclosed herein. However, by altering each of the variables, an optimum protocol can be derived for any plant species.

Further, the genetic properties engineered into the transgenic seeds and plants, plant parts, and/or plant cells of the present invention described herein can be passed on by sexual reproduction or vegetative growth and therefore can be maintained and propagated in progeny plants. Generally, maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as harvesting, sowing or tilling.

In addition to the stably transformed plants, plant parts, plant cells, yeast and bacterial cells provided herein, the invention further provides products produced from said stably transformed plant, plant cell, plant part, yeast cell or bacterial cell of the invention. In some embodiments, the product produced can include but is not limited to biofuel, food, drink, animal feed, fiber, commodity chemicals, cosmetics and/or pharmaceuticals.

Additionally provided herein are seeds produced from the stably transformed plants of the invention, wherein said seeds comprise in their genome a heterologous polynucleotide encoding a SOR from an archaeon species. In other embodiments, the invention provides seeds produced from the stably transformed plants and comprising in their genome a heterologous polynucleotide encoding a SOR and a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species. In further embodiments, seeds produced from the stably transformed plants and comprising in their genome a heterologous polynucleotide encoding a SOR from an archaeon species and a heterologous polynucleotide encoding a CO₂ transporter are provided. In still further embodiments, seeds produced from the stably transformed plants and comprising in their genome a heterologous polynucleotide encoding a SOR from an archaeon species, a heterologous polynucleotide encoding a CO₂ transporter and a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species are provided.

As used herein, the terms “nucleic acid,” “nucleic acid molecule,” “nucleotide sequence” and “polynucleotide” refer to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2'-hydroxy in the ribose sugar group of the RNA can also be made.

As used herein, the term “nucleotide sequence” refers to a heteropolymer of nucleotides or the sequence of these nucleotides from the 5' to 3' end of a nucleic acid molecule and includes DNA or RNA molecules, including cDNA, a DNA fragment, genomic DNA, synthetic (*e.g.*, chemically synthesized) DNA, plasmid DNA, mRNA, and anti-sense RNA, any of which can be single stranded or double stranded. The terms “nucleotide sequence” “nucleic acid,” “nucleic acid molecule,” “oligonucleotide” and “polynucleotide” are also used interchangeably herein to refer to a heteropolymer of nucleotides. Nucleic acid sequences provided herein are presented herein in the 5' to 3' direction, from left to right and are represented using the standard code for representing the nucleotide characters as set forth in the U.S. sequence rules, 37 CFR §§1.821 - 1.825 and the World Intellectual Property Organization (WIPO) Standard ST.25.

As used herein, the term “gene” refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, miRNA, and the like. Genes may or may not be capable of being used to produce a functional protein. Genes can include both coding and non-coding regions (*e.g.*, introns, regulatory elements, promoters, enhancers, termination sequences and 5' and 3' untranslated regions). A gene may be “isolated” by which is meant a nucleic acid molecule that is substantially or essentially free from components normally found in association with the nucleic acid molecule in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid molecule.

As used herein, the terms “fragment” when used in reference to a polynucleotide will be understood to mean a nucleic acid molecule or polynucleotide of reduced length relative to a reference nucleic acid molecule or polynucleotide and comprising, consisting essentially of and/or consisting of a nucleotide sequence of contiguous nucleotides identical or almost identical (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent.

As used herein, a “functional” polypeptide or “functional fragment” is one that substantially retains at least one biological activity normally associated with that polypeptide. In particular embodiments, the “functional” polypeptide or “functional fragment” substantially retains all of the activities possessed by the unmodified peptide. By “substantially retains” biological activity, it is meant that the polypeptide retains at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of

the biological activity of the native polypeptide (and can even have a higher level of activity than the native polypeptide). A “non-functional” polypeptide is one that exhibits little or essentially no detectable biological activity normally associated with the polypeptide (*e.g.*, at most, only an insignificant amount, *e.g.*, less than about 10% or even 5%). Thus, for
5 example, a functional fragment of an archaeon SOR polypeptide is a polypeptide that retains at least 50% or more SOR activity or a functional fragment of a CO₂ transporter polypeptide is a polypeptide that retains at least 50% or more CO₂ transporter activity.

An “isolated” nucleic acid molecule or nucleotide sequence or nucleic acid construct or double stranded RNA molecule of the present invention is generally free of nucleotide
10 sequences that flank the nucleic acid of interest in the genomic DNA of the organism from which the nucleic acid was derived (such as coding sequences present at the 5' or 3' ends). However, the nucleic acid molecule of this invention can include some additional bases or moieties that do not deleteriously or materially affect the basic structural and/or functional characteristics of the nucleic acid molecule.

Thus, an “isolated nucleic acid molecule” or “isolated nucleotide sequence” is a
15 nucleic acid molecule or nucleotide sequence that is not immediately contiguous with nucleotide sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. Accordingly, in one embodiment, an isolated nucleic acid includes some or all of the 5' non-
20 coding (*e.g.*, promoter) sequences that are immediately contiguous to a coding sequence. The term therefore includes, for example, a recombinant nucleic acid that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment), independent of other
25 sequences. It also includes a recombinant nucleic acid that is part of a hybrid nucleic acid molecule encoding an additional polypeptide or peptide sequence.

The term “isolated” can further refer to a nucleic acid molecule, nucleotide sequence, polypeptide, peptide or fragment that is substantially free of cellular material, viral material, and/or culture medium (*e.g.*, when produced by recombinant DNA techniques), or chemical
30 precursors or other chemicals (*e.g.*, when chemically synthesized). Moreover, an “isolated fragment” is a fragment of a nucleic acid molecule, nucleotide sequence or polypeptide that is not naturally occurring as a fragment and would not be found as such in the natural state. “Isolated” does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or nucleic acid in a form in which it can be used

for the intended purpose. In representative embodiments of the invention, an “isolated” nucleic acid molecule, nucleotide sequence, and/or polypeptide is at least about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% pure (w/w) or more. In other embodiments, an “isolated” nucleic acid, nucleotide sequence, and/or polypeptide indicates that at least about a 5-fold, 10-fold, 25-fold, 100-fold, 1000-fold, 10,000-fold, 100,000-fold or more enrichment of the nucleic acid (w/w) is achieved as compared with the starting material.

As used herein, “complementary” polynucleotides are those that are capable of hybridizing via 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. For example, the sequence “A-G-T” binds to the complementary sequence “T-C-A.” It is understood that two polynucleotides may hybridize to each other even if they are not completely or fully complementary to each other, provided that each has at least one region that is substantially complementary to the other.

The terms “complementary” or “complementarity,” as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. Complementarity between two single-stranded molecules may be “partial,” in which only some of the nucleotides bind, or it may be complete when total complementarity exists between the single stranded molecules either along the full length of the molecules or along a portion or region of the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

As used herein, the terms “substantially complementary” or “partially complementary” mean that two nucleic acid sequences are complementary at least at about 50%, 60%, 70%, 80% or 90% of their nucleotides. In some embodiments, the two nucleic acid sequences can be complementary at least at about 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of their nucleotides. The terms “substantially complementary” and “partially complementary” can also mean that two nucleic acid sequences can hybridize under high stringency conditions and such conditions are well known in the art.

As used herein, “heterologous” refers to a nucleic acid molecule or nucleotide sequence that either originates from another species or is from the same species or organism but is modified from either its original form or the form primarily expressed in the cell.

Thus, a nucleotide sequence derived from an organism or species different from that of the cell into which the nucleotide sequence is introduced, is heterologous with respect to that cell and the cell's descendants. In addition, a heterologous polynucleotide includes a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, e.g. present in a different copy number, and/or under the control of different regulatory sequences than that found in the native state of the nucleic acid molecule.

As used herein, the terms “transformed” and “transgenic” refer to any plant, plant part, plant cell, yeast cell or bacterial cell that contains all or part of at least one recombinant (e.g., heterologous) polynucleotide. In some embodiments, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. For the purposes of the invention, the term “recombinant polynucleotide” refers to a polynucleotide that has been altered, rearranged, or modified by genetic engineering. Examples include any cloned polynucleotide, or polynucleotides, that are linked or joined to heterologous sequences. The term “recombinant” does not refer to alterations of polynucleotides that result from naturally occurring events, such as spontaneous mutations, or from non-spontaneous mutagenesis followed by selective breeding.

The term “transgene” as used herein, refers to any nucleotide sequence used in the transformation of an organism. Thus, a transgene can be a coding sequence, a non-coding sequence, a cDNA, a gene or fragment or portion thereof, a genomic sequence, a regulatory element and the like. A “transgenic” organism, such as a transgenic plant, transgenic yeast, or transgenic bacterium, is an organism into which a transgene has been delivered or introduced and the transgene can be expressed in the transgenic organism to produce a product, the presence of which can impart an effect and/or a phenotype in the organism.

Different nucleotide sequences or polypeptide sequences having homology are referred to herein as “homologues.” The term homologue includes homologous sequences from the same and other species and orthologous sequences from the same and other species. “Homology” refers to the level of similarity between two or more nucleotide sequences and/or amino acid sequences in terms of percent of positional identity (i.e., sequence similarity or identity). Homology also refers to the concept of similar functional properties among different nucleic acids, amino acids, and/or proteins.

As used herein “sequence identity” refers to the extent to which two optimally aligned polynucleotide or polypeptide sequences are invariant throughout a window of alignment of

components, *e.g.*, nucleotides or amino acids. “Identity” can be readily calculated by known methods including, but not limited to, those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991).

As used herein, the term “substantially identical” or “corresponding to” means that two nucleotide sequences have at least 65%, 70%, 75%, 80%, 85%, 90% or 95% sequence identity. In some embodiments, the two nucleotide sequences can have at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity. Thus, for example, a homolog of a SOR from an archaeon species of this invention or a homolog of a CO₂ transporter of this invention can have at least about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to said SOR (see, *e.g.*, **Table 1**) or said aquaporin of this invention, respectively. In representative embodiments, an SOR useful with this invention can have about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a nucleotide sequence encoding a *P. furiosus* SOR polynucleotide (*e.g.*, **SEQ ID NO:1** or **SEQ ID NO:2**). In other embodiments, an SOR useful with this invention can have about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a *P. furiosus* SOR polypeptide encoded by the amino acid sequence of **SEQ ID NO:3** or **SEQ ID NO:4**.

Two nucleotide sequences can also be considered to be substantially identical when the two sequences hybridize to each other under stringent conditions. A nonlimiting example of “stringent” hybridization conditions include conditions represented by a wash stringency of 50% formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C. “Stringent hybridization conditions” and “stringent hybridization wash conditions” in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes* part I chapter 2 “Overview of principles of hybridization and the strategy of nucleic acid probe assays” Elsevier, New York (1993). In some representative embodiments, two nucleotide sequences considered to be substantially identical hybridize to each other under

highly stringent conditions. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH.

An “identity fraction” for aligned segments of a test sequence and a reference
5 sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, *i.e.*, the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. As used herein, the term “percent sequence identity” or “percent identity” refers to the percentage of identical
10 nucleotides in a linear polynucleotide sequence of a reference (“query”) polynucleotide molecule (or its complementary strand) as compared to a test (“subject”) polynucleotide molecule (or its complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). In some embodiments, “percent
15 identity” can refer to the percentage of identical amino acids in an amino acid sequence.

Optimal alignment of sequences for aligning a comparison window is well known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and optionally by computerized
20 implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG® Wisconsin Package® (Accelrys Inc., Burlington, Mass.). The comparison of one or more polynucleotide sequences may be to a full-length polynucleotide sequence or a portion thereof, or to a longer polynucleotide sequence. For purposes of this invention “percent identity” may also be determined using BLASTX version 2.0 for
25 translated nucleotide sequences and BLASTN version 2.0 for polynucleotide sequences.

The percent of sequence identity can be determined using the “Best Fit” or “Gap” program of the Sequence Analysis Software Package™ (Version 10; Genetics Computer Group, Inc., Madison, Wis.). “Gap” utilizes the algorithm of Needleman and Wunsch (Needleman and Wunsch, *J Mol. Biol.* 48:443-453, 1970) to find the alignment of two
30 sequences that maximizes the number of matches and minimizes the number of gaps. “BestFit” performs an optimal alignment of the best segment of similarity between two sequences and inserts gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (Smith and Waterman, *Adv. Appl. Math.*, 2:482-489, 1981, Smith *et al.*, *Nucleic Acids Res.* 11:2205-2220, 1983).

Useful methods for determining sequence identity are also disclosed in Guide to Huge Computers (Martin J. Bishop, ed., Academic Press, San Diego (1994)), and Carillo et al.

(*Applied Math* 48:1073(1988)). More particularly, preferred computer programs for determining sequence identity include but are not limited to the Basic Local Alignment

5 Search Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (NCBI) at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul *et al.*, NCBI, NLM, NIH;

(Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)); version 2.0 or higher of BLAST programs allows the introduction of gaps (deletions and insertions) into alignments; for peptide
10 sequence BLASTX can be used to determine sequence identity; and for polynucleotide sequence BLASTN can be used to determine sequence identity.

Accordingly, the present invention further provides nucleotide sequences having substantial sequence identity to the nucleotide sequences of the present invention (e.g., the polynucleotides encoding a SOR from an archaeon species or polynucleotides encoding a
15 CO₂ transporter). Substantial sequence similarity or identity means at least 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and/or 100% similarity or identity with another nucleotide sequence.

The following examples are not intended to be a detailed catalog of all the different ways in which the present invention may be implemented or of all the features that may be
20 added to the present invention. Persons skilled in the art will appreciate that numerous variations and additions to the various embodiments may be made without departing from the present invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

25 EXAMPLES

Example 1. Plants

Plants are continually challenged by environmental stresses that result in increased production of reactive oxygen species (ROS, e.g., superoxide and hydrogen peroxide). ROS can induce a switch from primary to secondary metabolism and can ultimately lead to plant
30 tissue death. Like other aerobic organisms, plants have ROS scavenging enzymes, such as superoxide dismutase (SOD), peroxidase and catalases that help prevent the production and buildup of toxic free radicals.

Pyrococcus furiosus is an extremophilic (hyperthermophile) species of archaea with optimum growth at 100°C. It is found in hydrothermal vents and is a strict anaerobe. *P.*

furiosus uses superoxide reductase (SOR – functional range of 4-100°C) rather than SOD to deal with ROS. Unlike SOD, the endogenous plant enzyme, SOR is more efficient in removing ROS and does so without producing oxygen (i.e. reducing the potential for further ROS generation). Thus, for example, transformation of a plant to express an archaeon SOR in the chloroplast can assist in the reduction of ROS, thereby protecting the transgenic plant's photosynthetic reaction centers, lowering O₂ content, which in turn helps to reduce photorespiration, and reduce expression of defense mechanisms that diminish photosynthetic electron flux.

Camelina sativa plants stably transformed with a heterologous polynucleotide encoding a *P. furiosus* SOR and expressing the SOR in the chloroplasts are assessed for protection of the photosynthetic apparatus and its surrounding membrane lipids from oxidative damage, reduced photosynthetic electron flux, and increased tolerance to abiotic stresses (e.g., drought, heat, high light). Transgenic plants in which SOR is targeted to the chloroplast, mitochondria, peroxisome, and/or cytosolic membrane are assessed for delayed senescence and increased abiotic stress tolerance and biomass production. Transgenic plants expressing *P. furiosus* SOR in cell walls are assessed for reduction in lignin polymerization and for an increased accessibility of cell wall cellulose to at least one cell wall degrading enzyme such as cellulase and hemicellulase. Exemplary vectors for transformation of plants are provided in **Fig. 1**.

Example 2. Yeast

Industrial yeast strains generate reactive oxygen species (ROS, e.g., superoxide and hydrogen peroxide) in response to fermentation product accumulation and metabolic flux. ROS can oxidatively damage cellular components and can ultimately lead to cell death. Like other facultative aerobic organisms, yeast have ROS scavenging enzymes, such as superoxide dismutase (SOD), peroxidase and catalases that help prevent the production and buildup of toxic free radicals. However, transformation of yeast with archaeal SOR (targeted to the mitochondria, cytosol or as a membrane associated protein) would help further protect yeast from the ROS generated by metabolic flux and fermentation product buildup (ex. ethanol). Exemplary vectors for transformation of yeast are provided in **Fig. 2**.

Example 3. Bacteria

Industrial bacterial strains, such as those used for biofuel production (cyanobacteria, *E. coli*, *Clostridium*), generate ROS in response to metabolic flux and biofuel molecule accumulation. ROS can irreversibly damage bacterial macromolecules and cell structures and

can ultimately lead to bacterial cell death. Transformation of bacteria with archaeal SOR (targeted either to the cytosol, to the periplasm, or as a membrane-associated protein) would aid in protecting the bacterial cells from ROS generated by metabolic flux and biofuel molecule accumulation. In some embodiments, when the SOR to be expressed in a bacterial cell is targeted to the periplasm, the periplasmic targeting protein can be encoded by the nucleotide sequence of atgaaacagagcaccattgcgaaagcgaaaaaccgctgctgtttaccccggtgaccaaagcg (SEQ ID NO:52) or the amino acid sequence of MKQSTIAKAKKPLLFTPVTKA (SEQ ID NO:48).

Example 4. Preparation of *P. furiosus* superoxide reductase polynucleotide for plant transformation

The gene encoding *P. furiosus* superoxide reductase (SOR) was amplified using polymerase chain reaction (PCR), *pfu* DNA polymerase and the indicated primers (forward primers; 5'-CAC CAT GAT TAG TGA AAC CAT AAG-3' (SEQ ID NO:53) for cloning into pENTR/D/TOPO and 5'-ATG ATT AGT GAA ACC ATA AG-3' (SEQ ID NO:54) for cloning into pCR8/GW/TOPO) and reverse primer; 5'-TCA CTC TAA AGT GAC TTC GTT TTC-3', SEQ ID NO:55) to amplify the coding region of SOR. The resulting amplification product was subcloned into pENTR/D/TOPO and pCR8/GW/TOPO entry vectors (Invitrogen, Carlsbad, CA) and then into pEG100, pEG103 and pEG104 destination vectors (Functional Genomics Division of the Department of Plant Systems Biology, Gent, Belgium) using LR recombination reactions according to the manufacturer's instructions (Invitrogen). The resulting constructs were as follows:

pEG103:SOR---C terminal GFP

pEG104:SOR---N terminal YFP

pEG100:SOR---no tags

pEG100:EGFP-SOR---N terminal fusion with EGFP

These above constructs enabled production of green fluorescence protein (GFP)-fusion-SOR proteins under the control of a CaMV 35S promoter in plants. Recombinant plasmids were transformed into *Agrobacterium tumefaciens* GV3101 using electroporation and then transformed into *Camelina* by vacuum infiltration of the inflorescences (Lu et al. *Plant Cell Reports* 27:273-278 (2008)). Four independent transformed lines were further selected. Stable expression of the transgene was monitored by RT-PCR and immunoblotting as described below.

SOR nucleotide and amino acid sequences:

(1) Nucleotide sequence of *P. furiosus* SOR: SEQ ID NO:1.

(2) Amino acid sequence of *P. furiosus* SOR:

5 Met I S E T I R S G D W K G E K H V P V I E Y E R E G E L V K V K V Q V G K E I P H
P N T T E H H I R Y I E L Y F L P E G E N F V Y Q V G R V E F T A H G E S V N G P N
T S D V Y T E P I A Y F V L K T K K K G K L Y A L S Y C N I H G L W E N E V T L E
Stop (SEQ ID NO:3)

10 (3) Nucleotide sequence of *P. furiosus* SOR variant: SEQ ID NO:2.

(4) Amino acid sequence of *P. furiosus* SOR variant:

Met I S E T I R S G D W K G E K H V P V I E Y E R E G E L V K V K V Q V G K E I P H
P N T T E H H I R Y I E L Y F L P E G E N F V Y Q V G R V E F T A H G E S V N G P N
T S D V Y T E P I A Y F V L K T K K K G K L Y A L S D C N I H G L W E N E V T L E
15 Stop (SEQ ID NO:4)

Targeting sequences:

(1) Chloroplast signal sequence: amino acid sequence of SEQ ID NO:5; nucleotide sequence of SEQ ID NO:6.

20 (2) Mitochondrial signal sequence: amino acid sequence of SEQ ID NO:7 or 9; nucleotide sequence of SEQ ID NO:8 or 10.

(3) Cell wall signal sequence: amino acid sequence of SEQ ID NO:11; nucleotide sequence of SEQ ID NO:12.

25 (4) Peroxisome signal sequence: amino acid sequence of SEQ ID NO:13; amino acid sequence of SEQ ID NO:14; nucleotide sequence of SEQ ID NO:15; amino acid sequence of SKL or nucleotide sequence of agcaaaactg.

(5) Dual signal sequence for mitochondria and chloroplast: amino acid sequence of SEQ ID NO:16; nucleotide sequence of SEQ ID NO:17.

30

Vectors for plant transformation

(1) **Exemplary constructs for chloroplast targeting**

(A) **Chloroplast targeted SOR** (pEG100:CTP- SOR): amino acid sequence of SEQ ID NO:18; nucleotide sequence of SEQ ID NO:19.

35 (B) **Chloroplast targeted SOR with EGFP as N terminal fusion**

pEG100:CTP-EGFP-SOR nucleotide sequence (chloroplast targeting peptide-enhanced green fluorescent protein-superoxide reductase (CTP-EGFP-SOR)): amino acid sequence of SEQ ID NO:20; nucleotide sequence of SEQ ID NO:21.

(C) **pEG100:CTP- SOR -EGFP Sequence:** amino acid sequence of **SEQ ID NO:22**;
nucleotide sequence of **SEQ ID NO:23**.

(D) **Chloroplast targeted SOR with yellow fluorescent protein (YFP) as N terminal fusion (CTP-YFP-SOR amino acid sequence):** amino acid sequence of **SEQ ID NO:24**;

5 nucleotide sequence of **SEQ ID NO:25**.

Maps of exemplary vectors for chloroplast transformation are provided in **Figs. 1A-C** (pEG100:CTP-SOR, pEG100:CTP-EGFP-SOR, and pEG100:CTP-SOR-EGFP, respectively). In each case, the vector includes *Bar* as a selection marker, 35S as the promoter, attR sites, the 3' sequences of the octopine synthase gene (OCS).

10 **Example 5. *Camelina sativa* transformation with chloroplast targeted SOR sequences**

Agrobacterium-mediated transformation was used to introduce the *P. furiosus* SOR into the chloroplasts, mitochondria, peroxisomes, and/or cell walls of *C. sativa* plants.

Constructs are provided in **Table 4**.

Table 4. Constructs used in transformation of Camelina.

Construct	NOTES	Entry vector	Destination vector
35S: SOR-GFP	GFP on C terminus	pCR8/GW/TOPO	pEG103→35S-GW-GFP-OCS-3'
35S:YFP-SOR	YFP on N terminus	pCR8/GW/TOPO	pEG104→35S-YFP-GW- OCS-3'
35S:eGFP-SOR	eGFP on N terminus	pENTR/D/TOPO	pEG100→35S-GW-OCS-3'
35S: CTP-SOR	No tags	pCR8/GW/TOPO	pEG100→35S-GW-OCS-3'
35S: SOR	No tags	pENTR:SOR	pEG100→35S-GW-OCS-3'
35S: CTP-egfp-SOR	CTP is in the N terminal side for targeting to chloroplasts	pCR8/GW/TOPO	pEG100→35S-GW-OCS-3'

15

Protocol for Transforming Camelina

Luria Broth (LB) medium for growing *Agrobacterium*

Infiltration medium:

1/2X MS salts

20

5% (w/v) Sucrose

0.044uM BAP

0.05% Silwet L-77

Procedure:

(1) Two days prior to transformation, a pre-culture of *Agrobacterium* carrying the appropriate binary vector is prepared by inoculating the *Agrobacterium* onto 3 ml LB medium including suitable antibiotics and incubating the culture at 28°C.

25

- (2) One day prior to transformation a larger volume of (150ml-300ml) LB medium is inoculated with at least 1ml of the preculture and incubated at 28°C for about 16-24 hrs.
- (3) Water plants prior to transformation.
- (4) On the day of transformation of the plant, *Agrobacterium* cells are pelleted by centrifugation at 6000 rpm for 10 min at room temperature (e.g., about 19°C to about 24°C).
- (5) The pellet is resuspended in 300-600 ml of infiltration medium (note: the infiltration medium is about double the volume used in the agro culture (about 150-300ml)).
- (6) The suspension solution is transferred to an open container that can hold the volume of infiltration medium prepared (300-600ml) in which plants can be dipped and which fits into a desiccator.
- (7) Place the container from (6) into a desiccator, invert a plant and dip the inflorescence shoots into the infiltration medium.
- (8) Connect the desiccator to a vacuum pump and evacuate for 5 min at 16-85kPa. (
- (9) Release the vacuum slowly.
- (10) After releasing vacuum, remove the plants and orient them into an upright position or on their sides in a plastic nursery flat, and place a cover over them for the next 24 hours.
- (11) The next day, the cover is removed, the plants rinsed with water and returned to their normal growing conditions (e.g., of about 22°C/18°C (day/night) with daily watering under about 250-400 μ E white light).
- (12) A week later the plants were transformed again, repeating steps 1-11.
- (13) The plants were watered on alternate days beginning after transformation for about 2-3 weeks and then twice a week for about another 2 weeks after which they were watered about once a week for about another 2 -3 weeks for drying.

Example 6. *Camelina sativa* transformation with sequences targeted to mitochondria, cell wall, cytosolic membrane, and/or peroxisome.

- (1) Exemplary targeting sequences for mitochondrial targeting: amino acid sequence of **SEQ ID NO:26**; nucleotide sequence of **SEQ ID NO:27**.
- (2) Exemplary targeting sequences for cell wall targeting: amino acid sequence of **SEQ ID NO:28**; nucleotide sequence of **SEQ ID NO:29**.
- (3) Exemplary targeting sequences for peroxisomal targeting.
 - (A) At the N terminus: amino acid sequence of **SEQ ID NO:30**; nucleotide sequence of **SEQ ID NO:31**.
 - (B) At the C terminus: amino acid sequence of **SEQ ID NO:32**; nucleotide sequence of **SEQ ID NO:33**.

(4) Exemplary targeting sequences for targeting to the cytosolic membrane:

CVVQ (SEQ ID NO:34); tgtgtcgtgcag (SEQ ID NO:35)

SOR plus the targeting target peptide sequence: amino acid sequence of SEQ ID NO:36; nucleotide sequence of SEQ ID NO:37.

5 The general motif for prenylation or farnesylation is C-terminal CaaX box motif on target proteins. C=cysteine; a=aliphatic amino acid; x=any amino acid. For plants, this motif most often is CVVQ (SEQ ID NO:34). This motif is required for specific posttranslational modifications (i.e. prenylation, farnesylation) that target the protein for association with the cytosolic side of the plasma membrane (the “inner” cytosolic side of the
10 cell) or the cytosolic side of the nuclear membrane.

(5) Exemplary construct for targeting to the mitochondria and chloroplast: amino acid sequence of SEQ ID NO:38; nucleotide sequence of SEQ ID NO:39.

Example 7. Analysis of transformed *C. sativa* plants.

(1) Verification of expression in the various plant organelles

15 *RT-PCR and pRT-PCR methods.*

RNA is isolated using the RNeasy kit (Qiagen), with an additional DNase I treatment to remove contaminating genomic DNA. Reverse transcription (RT) was carried out to generate cDNA using Omniscript reverse transcriptase enzyme (Qiagen). GFP-fused-SOR transcripts can be detected by PCR as described by Im *et al.*, (2005) using internal GFP
20 forward and gene specific primers (SOR reverse and actin specific primers), *APX* specific primers described in (Panchuk *et al. Plant Physiol* 129: 838–853 (2002) and *Zat12* specific primers (forward; 5' AACACAAACCACAAGAGGATCA 3', SEQ ID NO:56, and reverse; 5' CGTCAACGTTTTCTTGTTCCA 3', SEQ ID NO:57). Quantitative RT-PCR was carried out using Full Velocity SYBR-Green® QPCR Master Mix (Stratagene) on a MX3000P
25 thermocycler (Stratagene). Gene specific primers for select genes were designed with the help of AtRTPrimer, a database for generating specific RT-PCR primer pairs (Han and Kim, *BMC Bioinformatics* 7:179 (2006)). Relative gene expression data were generated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, *Methods* 25:402–408 (2001)) using the wild-type zero time point as the reference. PCR conditions were 1 cycle of 95°C for 10 min, 95°C for 15 s,
30 and 60°C for 30 s to see the dissociation curve, 40 cycles of 95°C for 1 minute for DNA denaturation, and 55°C for 30 s for DNA annealing and extension.

Immunoblotting (Western analysis for SOR detection)

Total protein extract is obtained from liquid N₂ frozen plants or seedlings grown as described by Weigel and Glazebrook, *Arabidopsis: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2002)). Protein concentration is quantified as described by Bradford (*Anal Biochem* 72: 248–254, (1976)). Protein is separated by 10 %
 5 (w/v) SDS-PAGE and detected with rabbit antibodies raised against *P. furiosus* SOR (at 1:2,000 dilution) or antibodies raised against HSP70, BiP, and CRT (at 1:1,000 dilution). Immunoreactivity is visualized with either horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies (Pierce, Rockford, IL).

SOR activity assay

10 Samples are ground with liquid nitrogen and lysed as described previously (Im *et al.*, *FEBS Lett* 579: 5521–5526 (2005)). Samples are centrifuged at 27,000g at 4 °C for 30 min and resulting supernatants are passed through a 0.45 micron filter unit to remove cellular debris. Extracts are dialyzed overnight in 50 mM phosphate buffer. To reduce plant SOD background activity of dialyzed samples, samples are heat-treated (heat-treated at 80°C for 15
 15 min) and centrifuged at 21,000g for 15 min. The heat treatments used are sufficient to inactivate some endogenous plant SOD activity, allowing for greater discrimination between SOD and SOR activity in the transgenic plants. To avoid leaf pigments and reduce loss of activity resulting from dialysis, roots are harvested from seedlings grown for 28 days or 42 days on agar plates in a growth chamber (8h light/16h dark).

20 The standard SOD/SOR assay is performed as described in Im *et al.* (*FEBS Lett* 579: 5521–5526 (2005)). One unit of SOD/SOR activity is defined as the amount of enzyme that inhibits the rate of reduction of cytochrome c by 50% (McCord and Fridovich, *J Biol Chem* 244: 6049–6055 (1969)).

(2) Reduction in ROS

H₂O₂ measurements (FOX assay)

25 A ferrous ammonium sulfate/xylenol orange (FOX) method is used to quantify H₂O₂ in plant extracts (Wolff, *Methods Enzymol* 233: 182–189, 1994)). The original FOX method is modified by addition of an acidification step where 1 ml of 25 mM H₂SO₄ was added to each sample to allow for precipitation of interfering substances (sugars, starches,
 30 polysaccharides) for 15 min on ice, and centrifuged at 9,700g, for 15 min, at 4°C. The cell free extract is collected and passed through a 0.45 µm-filter unit. 100 µl is added to 1 ml of the FOX reagent, mixed, and incubated at room temperature for 20 min. The concentration of H₂O₂ in the reagent is calibrated using absorbance at 240 nm and an extinction coefficient

of $43.6 \text{ M}^{-1} \text{ cm}^{-1}$. The concentration of H_2O_2 is measured in nmoles H_2O_2 per gram of fresh wt cells.

Ascorbate peroxidase (APX) activity assay

APX activity is determined as described previously (Nakano and Asada, *Plant Cell Physiol* 22:867–880, 1981). Fifty μg of the extract is used in a 3 ml APX assay and the reaction proceeds for 2 minutes. APX activity is expressed as μmol of ascorbate oxidized $(\text{mg protein})^{-1} \text{ min}^{-1}$. Additional confirmation of APX activity can be done by an in-gel assay as described by Panchuk *et al.* (*Plant Physiol* 129: 838–853 (2002)).

(3) Protection of the photosynthetic apparatus and its surrounding membrane lipids

To quantify the protection of the photosystems, leaf fluorescence and CO_2 fixation rates of fully expanded leaves is measured using a LiCOR system. The maximal photochemical efficiency of the PSII is calculated using the ratio F_v/F_m , where $F_v = F_m - F_0$ (Genty *et al.*, *Biochimica et Biophysica Acta (BBA) - General Subjects* 990: 87-92 (1989)). This is calculated from initial (F_0) and maximum fluorescence (F_m) as measured *in vivo* on the last fully expanded leaf pre-acclimatized to the dark for approximately 40 min. F_m can be estimated by applying a light saturating flash with an intensity of ca. $8,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

(4) Reduction in photorespiration

Reduction in photorespiration is determined by CO_2 fixation rates as described above using a LICOR system. Plants are exposed to atmospheric $\text{CO}_2:\text{O}_2$ mixtures (400ppm $\text{CO}_2/21\% \text{ O}_2$) or at saturating CO_2 concentrations (4000ppm/21% O_2) and their biomass, photosynthetic CO_2 fixation rates, chlorophyll fluorescence and chlorophyll content are quantified. Higher CO_2 fixation rates in the transgenic plants under limiting CO_2 compared to wild type and control plants indicate reduced photorespiratory activity.

(5) Increased disease resistance

Transgenic *Camelina sativa* plants comprising a heterologous polynucleotide encoding SOR from *P. furiosus* (e.g., **SEQ ID NO:1**, **SEQ ID NO:2**) were challenged with powdery mildew (*Erysiphe cichoracearum*). 3, 3-Diamino benzidine (DAB) staining method was used for *in situ* detection of hydrogen peroxide in the powdery mildew infected leaves (Thordal-Christensen *et al.* *Plant J* 11(6): 1187-1194 (1997)). DAB is prepared in water and protected from light. The two youngest leaves per plant and two plants per line (of the drought treated plants) were selected for the assay (WT, CS 9-5, CS 10-4). Once the

leaves were harvested from the plant, they are placed in the DAB solution upright such that the petiole is in the solution and vacuum infiltrated for 5 min. The multiwell plate with leaves in DAB solution is placed in a low light area. After 6 hrs of DAB treatment, the leaves were bleached in 95 % ethanol for 2hrs and then boiled in ethanol for 10 min. and then imaged.

5 Results are shown in **Figure 3**. For the purpose of imaging the leaves were infiltrated with DAB. Leaves of WT and two of the transgenic CTP-SOR plants were excised and treated with a solution of DAB for 6 hrs and assayed. Brown staining on the leaves is due to the formation of a brown polymerization product when H_2O_2 reacts with DAB. Powdery mildew lesions can be seen as brown haustoria on the leaves. **Figure 3** shows that the
10 transgenic plants inoculated with the powdery mildew had increased resistance (showed reduced growth of the pathogen on the plant and reduced symptoms).

 The above examples clearly illustrate the advantages of the invention. Although the present invention has been described with reference to specific details of certain
embodiments thereof, it is not intended that such details should be regarded as limitations
15 upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

THAT WHICH IS CLAIMED:

1. A stably transformed plant, plant part and/or plant cell, comprising a heterologous polynucleotide encoding a superoxide reductase (SOR) from an archaeon species, wherein said stably transformed plant, plant cell, and/or plant part has increased disease resistance.

2. The stably transformed plant, plant part and/or plant cell of claim 1, wherein said superoxide reductase is localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, and/or mitochondria of said stably transformed plant, plant cell, and/or plant part.

3. The stably transformed plant, plant part and/or plant cell of claim 2, wherein said superoxide reductase is a membrane associated protein of the chloroplast, the peroxisome, the cell wall, and/or mitochondria.

4. The stably transformed plant, plant part or plant cell of any of the preceding claims, wherein the archaeon species is a species from the genus *Pyrococcus*, a species from the genus *Thermococcus*, or a species from the genus *Archaeoglobus*.

5. The stably transformed plant, plant part or plant cell of claim 4, wherein the archaeon species is *Pyrococcus furiosus*.

6. The stably transformed plant, plant part or plant cell of claim 5, wherein the heterologous polynucleotide comprises a nucleotide sequence having substantial identity to the nucleotide sequence of **SEQ ID NO:1** or **SEQ ID NO:2**.

7. The stably transformed plant, plant part or plant cell of claim 5, wherein the heterologous polynucleotide encodes an amino acid sequence having substantial identity to an amino acid sequence of **SEQ ID NO:3** or **SEQ ID NO:4**.

8. The stably transformed plant, plant part or plant cell of any one of the preceding claims, wherein the plant, plant part, or plant cell, further comprises a heterologous polynucleotide encoding a CO₂ transporter.

9. The stably transformed plant, plant part or plant cell of any one of the preceding claims, wherein the plant, plant part, or plant cell comprises a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species, wherein the rubrerythrin reductase is co-localized with the SOR in said transformed plant, plant cell, plant part, yeast cell or bacterial cell.

10. The stably transformed plant, plant part or plant cell of claim 9, wherein the rubrerythrin reductase is encoded by a nucleotide sequence that encodes the amino acid sequence of **SEQ ID NO:50**.

11. The stably transformed plant, plant part or plant cell of any one of the preceding claims, wherein the superoxide reductase, rubrerythrin reductase and/or CO₂ transporter are each operably associated in fusion with a targeting sequence.

12. A seed of the stably transformed plant of any one of the preceding claims, wherein the seed comprises in its genome a heterologous polynucleotide encoding superoxide reductase from an archaeon species.

13. The seed of claim 12, wherein the seed comprises in its genome a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species.

14. The seed of claim 12 or 13, wherein the seed comprises in its genome a heterologous polynucleotide encoding a CO₂ transporter.

15. A product produced from the stably transformed plant, plant part, or plant cell of any one of the preceding claims.

16. The product of claim 15, wherein the product is biofuel, food, drink, animal feed, fiber, and/or pharmaceuticals.

17. A stably transformed plant, plant part, plant cell, yeast cell or bacterial cell comprising a first heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a second heterologous polynucleotide encoding a CO₂ transporter.

18. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell of claim 17, wherein said superoxide reductase is localized to the cytosolic membrane, the chloroplast, the peroxisome, the cell wall, mitochondria, periplasm and/or as a membrane associated protein of said transformed plant, plant cell, plant part, yeast cell or bacterial cell

5

19. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell of claim 17 or claim 18, wherein the archaeon species is a species from the genus *Pyrococcus*, a species from the genus *Thermococcus*, or a species from the genus *Archaeoglobus*.

10

20. The stably transformed plant, plant cell, plant part, yeast cell or bacterial cell of any one of claims 17 to 19, wherein the archaeon species is *Pyrococcus furiosus*.

21. The stably transformed plant, plant cell, plant part, yeast cell or bacterial cell of any one of claims 17 to 20, wherein the first heterologous polynucleotide comprises a nucleotide sequence having substantial identity to the nucleotide sequence of **SEQ ID NO:1** or **SEQ ID NO:2**.

22. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell of any one of claims 17 to 21, wherein the second heterologous polynucleotide encoding a CO₂ transporter is:

(a) a nucleotide sequence having substantial identity to the nucleotide sequence of **SEQ ID NO:58**, **SEQ ID NO:60** or **SEQ ID NO:62** and/or

(b) a nucleotide sequence that encodes an amino acid sequence having substantial identity to an amino acid sequence of **SEQ ID NO:59**, **SEQ ID NO:61** and/or **SEQ ID NO:62**.

23. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell, of any one of claims 17 to 22, wherein the second heterologous polynucleotide encodes an amino acid sequence having substantial identity to an amino acid sequence of **SEQ ID NO:59**, **SEQ ID NO:61** and/or **SEQ ID NO:62**.

24. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell, of any one of claims 17 to 23, wherein the plant, plant cell, plant part, yeast cell or bacterial

cell comprises a third heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species, wherein the rubrerythrin reductase is co-localized with the SOR in said transformed plant, plant cell, plant part, yeast cell or bacterial cell.

5 25. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell, of claim 24, wherein the rubrerythrin reductase is encoded by a nucleotide sequence that encodes the amino acid sequence of **SEQ ID NO:50**.

10 26. The stably transformed plant, plant part, plant cell, yeast cell or bacterial cell, of any of any one of claims 17-25, wherein the superoxide reductase, rubrerythrin reductase and/or CO₂ transporter are each operably associated (in fusion) with a targeting sequence.

15 27. A method of increasing disease resistance in a plant, plant cell, or plant part, comprising:
introducing into said plant, plant cell, or plant part a heterologous polynucleotide encoding a superoxide reductase from an archaeon species to produce a stably transformed plant, plant cell, or plant part, thereby producing a plant, plant part, or plant cell having increased disease resistance as compared to a control.

20 28. The method of claim 27, wherein said superoxide reductase is expressed and localized to the chloroplast, the cell wall, mitochondria and/or as a membrane associated protein of said stably transformed plant, plant cell, or plant part.

25 29. The method of claim 27 or claim 28, wherein the archaeon species is *Pyrococcus furiosus*.

30 30. The method of claim 29, wherein the heterologous polynucleotide encoding a superoxide reductase from *Pyrococcus furiosus* is a nucleotide sequence having substantial identity to a nucleotide sequence of **SEQ ID NO:1** and/or **SEQ ID NO:2**.

31. The method of claim 29, wherein the heterologous polynucleotide encoding a superoxide reductase from *Pyrococcus furiosus* encodes an amino acid sequence substantial identity to an amino acid sequence of **SEQ ID NO:3** and/or **SEQ ID NO:4**.

32. The method of any one of claims 27-31, wherein the plant, plant cell, plant part, yeast cell or bacterial cell further comprises a heterologous polynucleotide encoding a polypeptide having the enzyme activity of a CO₂ transporter

5 33. The method of any one of claims 27-32, wherein the plant, plant cell, plant part, yeast cell or bacterial cell comprises a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species, wherein the rubrerythrin reductase is co-localized with the SOR in said transformed plant, plant cell, plant part, yeast cell or bacterial cell.

10 34. The method of claim 33, wherein the rubrerythrin reductase is encoded by a nucleotide sequence that encodes the amino acid sequence of **SEQ ID NO:50**.

15 35. A stably transformed plant, plant cell and/or plant part produced by the method of any one of claims 27-34.

20 36. A seed of the stably transformed plant of claim 35, wherein the seed comprises in its genome a heterologous polynucleotide encoding a superoxide reductase from an archaeon species.

25 37. A seed of the stably transformed plant of claim 35, wherein the seed comprises in its genome a heterologous polynucleotide encoding a superoxide reductase from an archaeon species and a heterologous polynucleotide encoding a rubrerythrin reductase from an archaeon species, and optionally a heterologous polynucleotide encoding a CO₂ transporter.

38. A product produced from the stably transformed plant, plant cell, or plant part of claim 35 or the seed of claim 36 or claim 37.

30 39. The product of claim 38, wherein the product is biofuel, food, drink, animal feed, fiber, and/or pharmaceuticals.

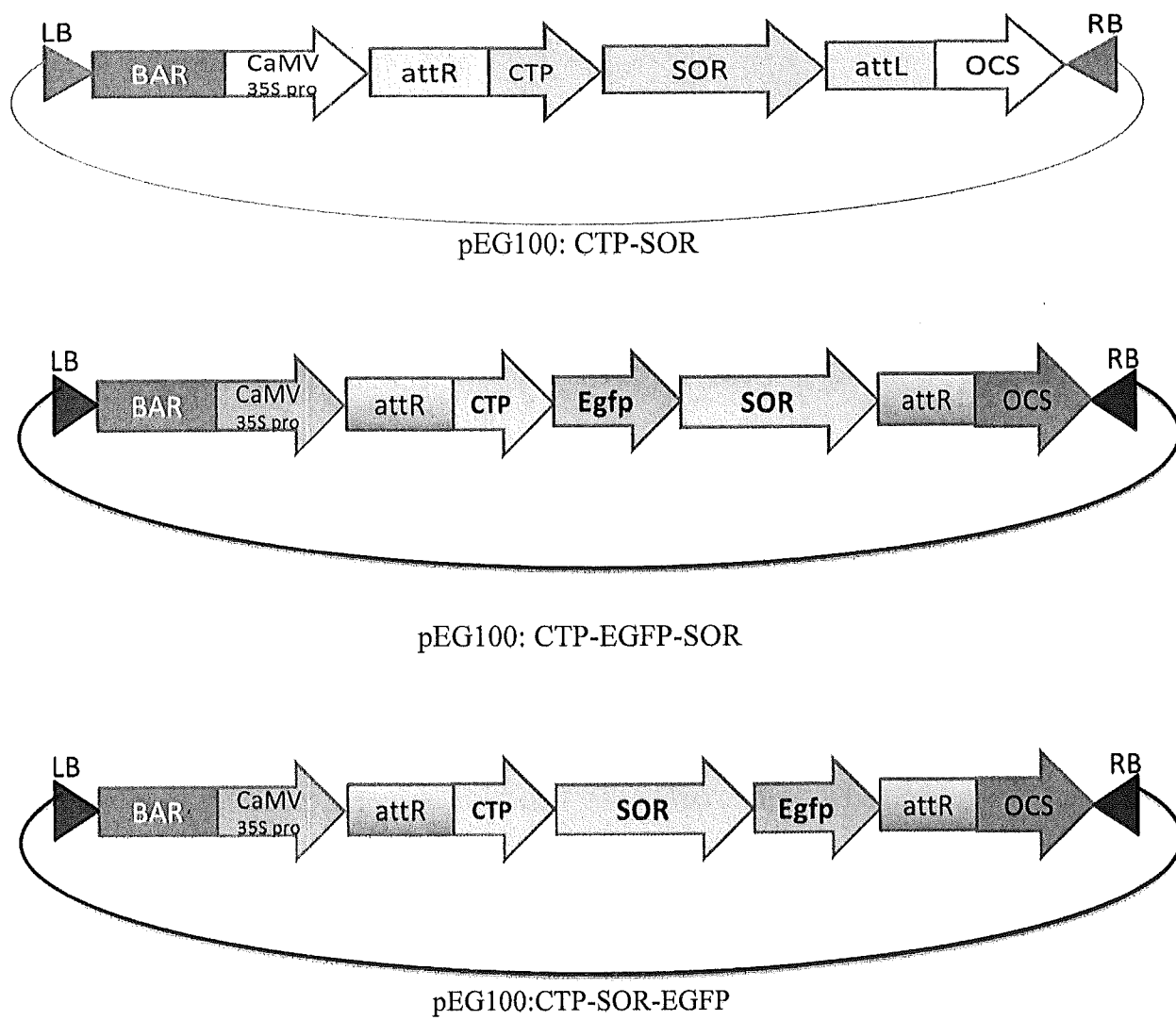
Fig. 1.

Fig. 2.

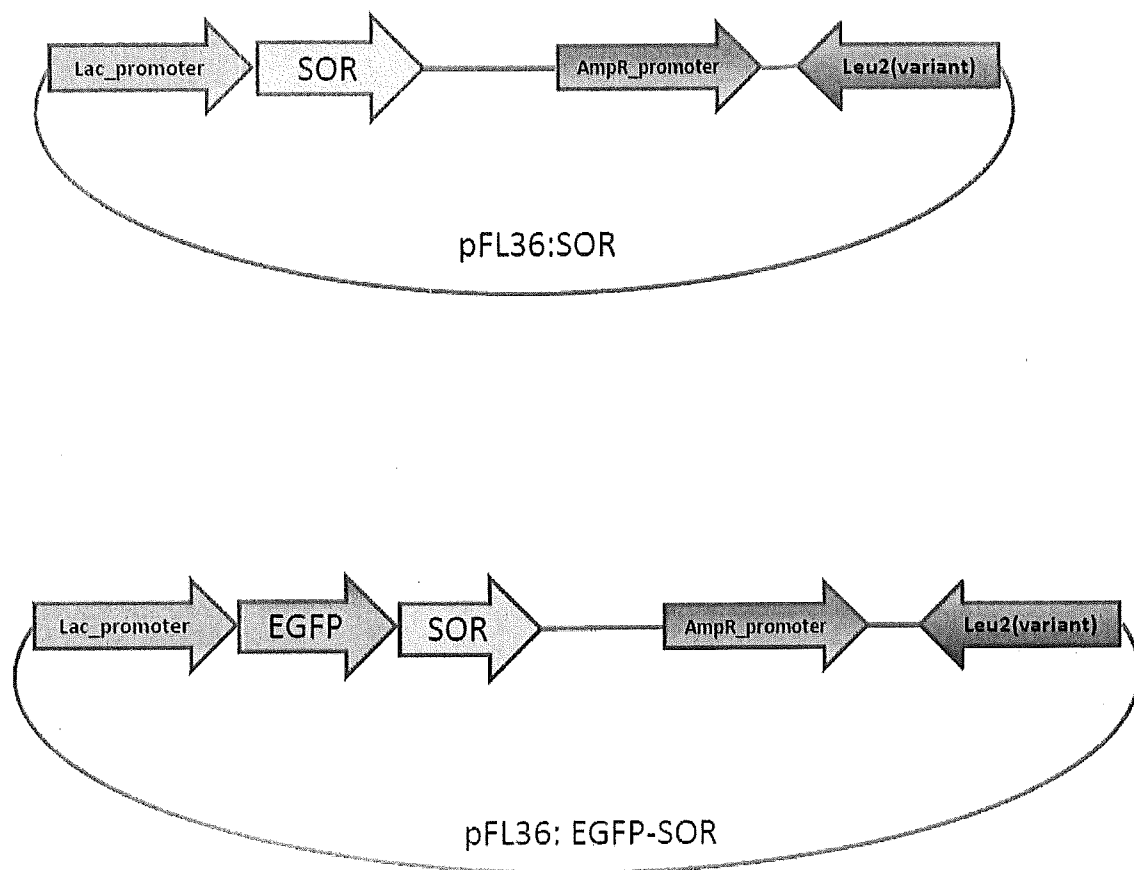
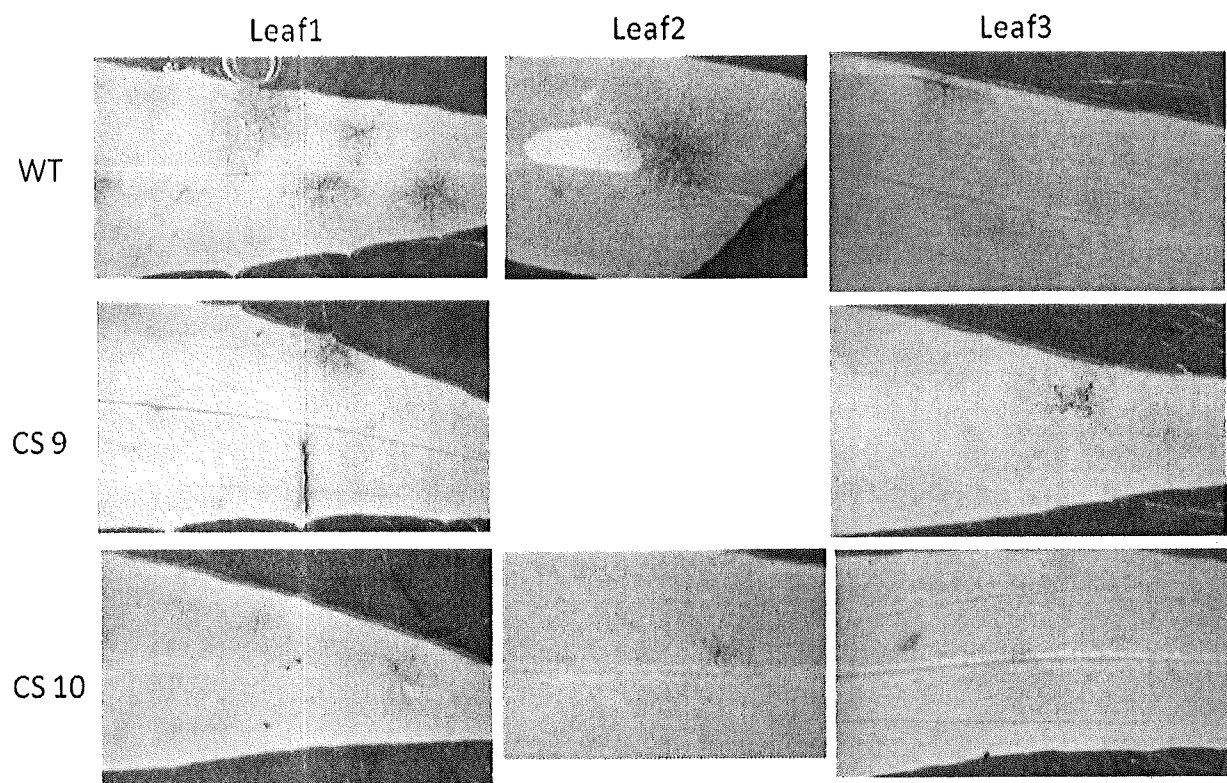


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/043393

A. CLASSIFICATION OF SUBJECT MATTER		
<i>C12N 15/53 (2006.01)</i> <i>C12N 9/02 (2006.01)</i> <i>A01H 1/00 (2006.01)</i>		
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 15/53, 9/02, A01H 1/00		
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 practicable, search terms used)		
CIPO, DEPATISNET, DWPI, EAPATIS, EMBL, ESP@CENET, KIPRIS, PAJ, PubMed, PatSearch (RUPTO internal), SCIEDIRECT, SIPO, USPTO		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	US 2006/0288446 A1 (VISHNEVETSKY J. et al) 21.12.2006, paragraphs [0003], [0011]-[0014], [0097]	15, 16, 38, 39 1-8, 11-12, 14, 17-19, 27-31, 35, 36 9, 10, 13, 37
Y	NIVIERE V. et al. Discovery of superoxide reductase: an historical perspective, J Biol Inorg Chem, 2004, Vol. 9, pp.119-123, especially abstract, p.119, 122	1-8, 11-12, 14, 17-19, 27-31, 35, 36
Y	MIKYOUNG LEE JI. Superoxide Reductase from the Hyperthermophilic Archaeon <i>Pyrococcus furiosus</i> : its Function, Regulation, and Biotechnological Applications, a dissertation submitted to the Graduate faculty of North Carolina State University, 2007, abstract, p.23-24, 120-122	1-8, 11-12, 14, 17-19, 27-31, 35, 36
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents:	“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
“A” document defining the general state of the art which is not considered to be of particular relevance	“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
“E” earlier document but published on or after the international filing date	“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
“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)	“&”	document member of the same patent family
“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		
Date of the actual completion of the international search	Date of mailing of the international search report	
03 October 2014 (03.10.2014)	30 October 2014 (30.10.2014)	
Name and mailing address of the ISA/RU: FIPS, Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer O.Tulinova Telephone No. 495 531 65 15	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/043393

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☒ on paper
☒ in electronic form
 - b. (time)
☒ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/043393

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.: 20-26, 32-34
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:

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 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.:

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/043393

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE GenBank: AF156097.1, 20.12.1999	6, 17-19, 30, 31
Y	DATABASE GenPept: WP_011012425.1, 15.05.2013	7, 17-19, 30, 31
Y	WO 2011/021190 A1 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM LTD et al) 24.02.2011, SEQ ID NO:1, claims 1, 2	8, 14, 17-29