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      10      20      30      40      50      60
BirA protein [Escherichia coli] -----MKDNT VPLKLIALLA NGEFHSGEQL GETLGMSSRAA INKHQTLRD WGVVETVPG
AF414937_1 Holocarboxylase Synthetase 1 MEAVRSTTTL SNFHLMLIV LRSKLPLRL SFSTASAME SDASCSLVLC GKSSVETVA

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
      70      80      90     100     110     120
BirA protein [Escherichia coli] KGVSLPEPIQ LIN-----AKQLTG QLD-----GGSVAVLPVI
AF414937_1 Holocarboxylase Synthetase 1 KGLKNKNSLK LFDNTRVSLI LESEAKNLVK DDNSFNLSL FMNSIITHRF GRFLIWSRL

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
     130     140     150     160     170     180
BirA protein [Escherichia coli] DSTNQYLLDR IGEKSGDAC VAETQAGRG RRGKWFSPF GANLYLSMFV RLEQGPAAAI
AF414937_1 Holocarboxylase Synthetase 1 SSTHDVSHN FSELPVGSVC VTDIQFKGRG RTKNVWESPK GCLMYS-FTL EMEDGRVPL

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
     190     200     210     220     230     240
BirA protein [Escherichia coli] GLSLVIGIVM AEVLKELGAD K-----VRV KWFNDLYLD REKLIGILVEL TGTGDAQAQI
AF414937_1 Holocarboxylase Synthetase 1 -IQYVVSIAV TEAVKDVCDK KGLPEIDVKI KWFNDLYVNG LKVGGLCTS TYRS-KKSNV

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
     250     260     270     280     290     300
BirA protein [Escherichia coli] VIGAGINMAM RRVEESVNG SWITLQKAGI NIDNTLAAM LIRKELAALE LFEQGLAP-
AF414937_1 Holocarboxylase Synthetase 1 SVGVGLNDVN G-QPTTCLNA VLKGNAPESN LLKREILGA FFHKFEKFFD LPMQGSFSL

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
     310     320     330     340     350     360
BirA protein [Escherichia coli] ---YLSWKEK LDFNFPVK LIIGKEIPG ISRGIDKQGA LLLQDGS---LTKPMWAGE
AF414937_1 Holocarboxylase Synthetase 1 KELYRTWLR SEQRVIVEDK VEDQVQGVV TIQGLTSSGY LLAVGDNDQM YELHFDGNST

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
     370
BirA protein [Escherichia coli] ISLRSAEK--
AF414937_1 Holocarboxylase Synthetase 1 DFFKGLVRKK I

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Fig. 1

(57) Abstract: This invention relates to methods for increasing carbon fixation, increasing biomass production and/or increasing abiotic stress tolerance in a plant comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotides are from a bacterial and/or an archaeal species. Further provided are plants, plant parts and plant cells produced by the methods of the invention.

METHODS AND COMPOSITIONS FOR ENHANCED BIOMASS PRODUCTION AND INCREASED ABIOTIC STRESS TOLERANCE

STATEMENT OF GOVERNMENT SUPPORT

5 This invention was made with government support under Grant No. DE-AR0000207 awarded by the United States Department of Energy (DOE). The United States government has certain rights in this invention.

STATEMENT OF PRIORITY

10 This application claims the benefit, under 35 U.S.C. § 119 (e), of U.S. Provisional Application No. 62/194,550, filed on July 20, 2015, the entire contents of which is incorporated by reference herein.

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 5051-884PR_ST25.txt, 66,777 bytes in size, generated on July 20, 2015 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 methods for increasing carbon fixation and biomass production in plants as well as for increasing abiotic stress tolerance.

BACKGROUND

25 All life depends on photosynthetic carbon fixation in which CO₂ is converted to organic compounds in the presence of water and light. However, this is an inefficient process, particularly in C₃ plants, because of a competing process called photorespiration. Photorespiration results in the release of about a quarter of the carbon that is fixed by photosynthesis. The inefficiency of C₃ photosynthesis is largely due to the enzyme ribulose-
30 1,5-bisphosphate carboxylase oxygenase (Rubisco) that catalyzes two competing reactions, carboxylation and oxygenation. Carboxylation leads to net fixed carbon dioxide and oxygenation utilizes oxygen and results in a net loss of carbon. The relative concentrations of carbon dioxide and oxygen and the temperature as well as water availability determine which reaction occurs or dominates. Thus, C₃ plants do not grow efficiently in hot and/or dry areas

because, as the temperature increases, Rubisco incorporates more oxygen. Some plants, such as C₄ and CAM (Crassulacean acid metabolism) plants, have developed mechanisms that reduce the effect of photorespiration by more efficiently delivering carbon dioxide to Rubisco, thereby outcompeting the oxygenase activity.

5

SUMMARY OF THE INVENTION

This invention is directed to methods for improving the efficiency of CO₂ fixation, increasing biomass production, and/or increasing abiotic stress tolerance in plants.

Thus, in one aspect, the present invention provides a method for increasing carbon
10 fixation and/or increasing biomass production in a plant, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species.

In another aspect of the invention, the present invention provides a method for
15 increasing carbon fixation and/or increasing biomass production in a plant, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species.

In still another aspect, a method for increasing carbon fixation and/or increasing
20 biomass production in a plant is provided, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotides are from a bacterial and/or an archaeal species.

25 In a further aspect, a method for increasing abiotic stress tolerance in a plant is provided, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species.

30 In another aspect, a method for increasing abiotic stress tolerance in a plant is provided, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species.

In an additional aspect of the invention, a method for increasing abiotic stress tolerance in a plant is provided, comprising: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotides are from a bacterial and/or an archaeal species.

In a further aspect, the present invention provides stably transformed plants, plant parts and/or plant cells, seeds from said stably transformed plants, and crops comprising said stably transformed plants.

In additional aspects, the present invention provides products produced from the transformed plants, plant parts and/or plant cells, seeds and/or crops of this invention.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows an amino acid sequence alignment for BirA Biotin Protein Ligase from *E. coli* and Holocarboxylase Synthetase from *Arabidopsis*.

Fig. 2 shows construct #3 consisting of p_CAMBIA_2300 and expression regions for BirA Biotin Protein Ligase, HyTh-Fdx ferredoxin, and the Basta resistance gene (BAR).

Fig. 3 shows T1 seedlings transformed with Construct #3 (**Fig. 2**) at 21 days after planting (DAP), C3 T1 seedlings shown in panel A measured 18 cm, compared to 9 cm for wild-type shown in panel B).

DETAILED DESCRIPTION

The present invention now will be described hereinafter with reference to the accompanying drawings and examples, in which embodiments of the invention are shown. 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 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 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.

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, means 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
5 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
10 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."

"Abiotic stress" or "environmental stress" as used herein means any outside, nonliving, physical or chemical factors or conditions that can have harmful effects on a plant. Thus, in some embodiments of the invention, an abiotic or environmental stress
15 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). Harmful effects due on a plant to abiotic stress can include, but are not limited to, reduced growth or size of a plant or plant part, reduced fruit and/or seed set, increased floral abortion, increased fruit drop, reduction in the number of plants or parts thereof,
20 and/or a reduction in crop yield and quality as compared to a plant or plants not experiencing abiotic stress.

"Complement" as used herein can mean 100% complementarity or identity with the comparator nucleotide sequence or it can mean less than 100% complementarity (e.g., about
25 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and the like, complementarity).

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "5'-A-G-T-3'" binds to the complementary sequence "3'-A-C-T-5'." Complementarity between two single-stranded molecules may be "partial," in which
30 only some of the nucleotides bind, or it may be complete when total complementarity exists between 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 “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
 5 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), miRNA, antisense RNA, ribozymes, RNA aptamers, and the like.

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
 10 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.*, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 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
 15 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”
 20 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%, 91%, 92%, 93%, 94%, 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
 25 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%).

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
 30 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.

“Genome” as used herein can refer to the nuclear genome, the chloroplast genome, the mitochondrial genome and/or a plasmid genome.

5 A “heterologous” or a “recombinant” nucleotide sequence is a nucleotide sequence not naturally associated with a host cell into which it is introduced, including non-naturally occurring multiple copies of a naturally occurring nucleotide sequence.

Different nucleic acids or proteins having homology are referred to herein as “homologues.” The term homologue includes homologous sequences from the same and
10 other species and orthologous sequences from the same and other species. “Homology” refers to the level of similarity between two or more nucleic acid 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 or proteins. Thus, the compositions and methods of the invention further comprise
15 homologues to the nucleotide sequences and polypeptide sequences of this invention. “Orthologous,” as used herein, refers to homologous nucleotide sequences and/ or amino acid sequences in different species that arose from a common ancestral gene during speciation. A homologue of a nucleotide sequence of this invention has a substantial sequence identity (e.g., at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%,
20 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and/or 100%) to said nucleotide sequence of the invention.

As used herein, hybridization, hybridize, hybridizing, and grammatical variations thereof, refer to the binding of two complementary nucleotide sequences or substantially complementary sequences in which some mismatched base pairs are present. The conditions
25 for hybridization are well known in the art and vary based on the length of the nucleotide sequences and the degree of complementarity between the nucleotide sequences. In some embodiments, the conditions of hybridization can be high stringency, or they can be medium stringency or low stringency depending on the amount of complementarity and the length of the sequences to be hybridized. The conditions that constitute low, medium and high
30 stringency for purposes of hybridization between nucleotide sequences are well known in the art (See, e.g., Gasiunas et al. (2012) *Proc. Natl. Acad. Sci.* 109:E2579-E2586; M.R. Green and J. Sambrook (2012) *Molecular Cloning: A Laboratory Manual*. 4th Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Two nucleotide sequences can also be considered to be substantially complementary 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 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 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 identity” can refer to the percentage of identical amino acids in an amino acid sequence.

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).

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 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 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 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 Search Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (e.g., NCBI) at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul *et al.*, e.g., 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 sequence BLASTX can be used to determine sequence identity; and for polynucleotide sequence BLASTN can be used to determine sequence identity.

The terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof), as used herein, describe an elevation in, for example, carbon fixation, biomass production, an elevation in CO₂ uptake, or an increase in abiotic stress tolerance in a plant, plant part or plant cell. This increase can be observed by comparing the increase in the plant, plant part or plant cell transformed with, for example, a heterologous polynucleotide encoding a bacterial or archaeal ferredoxin polypeptide and/or a biotin ligase polypeptide as compared to an appropriate control (e.g., the same organism (e.g., the same species of plant, plant part or plant cell) lacking (i.e., not transformed with) said heterologous polynucleotide(s)). 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 and/or plant cell that does not comprise said heterologous polynucleotide encoding a bacterial or archaeal ferredoxin polypeptide and/or a bacterial or archaeal biotin ligase polypeptide).

“Increased biomass production” as used herein refers to a transformed plant or plant part having a greater dry weight over the entire plant or any organ of the plant (leaf, stem, roots, seeds, seed pods, flowers, etc), increased plant height, leaf number, and/or seed number or increased root volume compared to the native or wild type (e.g., a plant, plant part that is not transformed with the heterologous polynucleotides of the invention (e.g., heterologous polynucleotides encoding a ferredoxin polypeptide and/or a biotin ligase polypeptide).

“Increased biomass production” can also refer to a greater dry weight of cells (e.g., tissue culture, cell suspension (e.g., algal culture) and the like) as compared to cells not transformed with the heterologous polynucleotides of the invention.

“Increased carbon fixation” as used herein refers to a greater conversion of CO₂ to organic carbon compounds in a transgenic plant (e.g., a plant, plant part that is not transformed with the heterologous polynucleotides of the invention (e.g., a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding biotin ligase polypeptide)) when compared to the native or wild type (e.g., not transformed with said heterologous polynucleotides). “Increased carbon fixation” can be measured by analyzing CO₂ fixation rates using a Licor System or radiolabeled ¹⁴CO₂ or by quantifying dry biomass. Increased carbon fixation can also occur for transformed cells (e.g.,

tissue culture, cell suspension (e.g., algal culture), and the like) as compared to cells not transformed with the heterologous polynucleotides of the invention.

In some embodiments, the recombinant nucleic acids molecules, nucleotide sequences and polypeptides of the invention are “isolated.” An “isolated” nucleic acid molecule, an “isolated” nucleotide sequence or an “isolated” polypeptide is a nucleic acid molecule, nucleotide sequence or polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule, nucleotide sequence or polypeptide may exist in a purified form that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In representative embodiments, the isolated nucleic acid molecule, the isolated nucleotide sequence and/or the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more pure.

In other embodiments, an isolated nucleic acid molecule, polynucleotide or polypeptide may exist in a non-native environment such as, for example, a recombinant host cell. Thus, for example, with respect to nucleotide sequences, the term “isolated” means that it is separated from the chromosome and/or cell in which it naturally occurs. A polynucleotide is also isolated if it is separated from the chromosome and/or cell in which it naturally occurs in and is then inserted into a genetic context, a chromosome and/or a cell in which it does not naturally occur (e.g., a different host cell, different regulatory sequences, and/or different position in the genome than as found in nature). Accordingly, the polynucleotides and their encoded polypeptides are “isolated” in that, by the hand of man, they exist apart from their native environment and therefore are not products of nature, however, in some embodiments, they can be introduced into and exist in a recombinant host cell.

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.

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 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 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.

Any plant (or groupings of plants, for example, into a genus or higher order classification) can be employed in practicing this 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, 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 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, 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 genus including, but not limited to, the genus of *Camelina*, *Sorghum*, *Gossypium*, *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* or *Gossypium*.

In other embodiments, a plant, plant part or plant cell can be from a species including, but not limited to, the species of *Camelina alyssum* (Mill.) Thell., *Camelina microcarpa* Andr. ex DC., *Camelina rumelica* Velen., *Camelina sativa* (L.) Crantz, *Sorghum bicolor* (e.g., *Sorghum bicolor* L. Moench), *Gossypium hirsutum*, *Brassica oleracea*, *Brassica rapa*, *Brassica napus*, *Raphanus sativus*, *Armoracia rusticana*, *Allium sativum*, *Allium cepa*, *Populus grandidentata*, *Populus tremula*, *Populus tremuloides*, *Prunus serotina*, *Prunus pensylvanica*, *Castanea dentata*, *Populus balsamifer*, *Populus deltoids*, *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 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 further embodiments, a plant and/or plant cell can be an alga or alga 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 *Achnantheidium*, *Actinella*, *Nitzschia*, *Nupela*, *Geissleria*, *Gomphonema*, *Planothidium*, *Halamphora*, *Psammothidium*, *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.

As used herein, the terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” “suppress,” and “decrease” (and grammatical variations thereof) means diminished, a decrease in, or a diminution in, for example, plant size, as a response to abiotic stress (e.g., 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%, or 100%, or any range therein, as compared to a control). ”

As used herein, the term “substantially identical” means that two nucleotide sequences have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity. Thus, for example, a homolog of a polynucleotide of the invention can have at least about 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to, for example, a polynucleotide encoding a ferredoxin polypeptide and/or a biotin ligase polypeptide.

As used herein, the terms “transformed” and “transgenic” refer to any plant, plant part, and/or plant 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.

The term “transformation” as used herein refers to the introduction of a heterologous polynucleotide into a cell. Transformation of a plant, plant part, and/or plant 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.

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, 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 minichromosome. The phrase “a stably transformed plant, plant part, and/or plant cell expressing said one or more polynucleotide sequences” and similar phrases used herein, means that the stably transformed plant, plant part, and/or plant cell comprises the one or more polynucleotide sequences and that said one or more polynucleotide sequences are functional in said stably transformed plant, plant part, and/or plant cell.

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.

Accordingly, the present invention is directed to compositions and methods for increasing carbon fixation, biomass production and/or abiotic stress tolerance in a plant, plant cell and/or plant part by introducing in the plant, plant cell and/or plant part a heterologous polynucleotide that encodes a ferredoxin polypeptide and/or a heterologous polynucleotide that encodes a biotin ligase polypeptide as described herein.

Thus, in some embodiments, the present invention provides a method for increasing carbon fixation and/or increasing biomass production in a plant, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotide, thereby increasing carbon fixation and/or increasing biomass production in said stably transformed plant, plant part, and/or plant cell as compared to a control (*e.g.*, a plant, plant part, and/or plant cell not comprising said heterologous polynucleotide), wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species.

In another embodiment, a method for increasing carbon fixation and/or increasing biomass production in a plant is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotide, thereby increasing carbon fixation and/or increasing biomass production in said stably transformed plant, plant part, and/or plant cell as compared to a control, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species.

In additional embodiments, a method for increasing carbon fixation and/or increasing biomass production in a plant is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase

polypeptide to produce a stably transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotides, thereby increasing carbon fixation and/or increasing biomass production in said stably transformed plant, plant part, and/or plant cell as compared to a control, wherein the heterologous polynucleotide encoding a ferredoxin polypeptide and the
5 heterologous polynucleotide encoding a biotin ligase polypeptide are from a bacterial and/or an archaeal species.

Further provided is a method for increasing abiotic stress tolerance in a plant, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce
10 a stably transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotide, thereby increasing abiotic stress tolerance in said stably transformed plant, plant part, and/or plant cell as compared to a control, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species.

In some embodiments, a method for increasing abiotic stress tolerance in a plant is
15 provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotide, thereby increasing abiotic stress tolerance in said stably transformed plant, plant part, and/or plant cell as compared to a control, wherein the
20 heterologous polynucleotide is from a bacterial and/or an archaeal species.

In some embodiments, a method for increasing abiotic stress tolerance in a plant is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably
25 transformed plant, plant part, and/or plant cell expressing said heterologous polynucleotides, thereby increasing abiotic stress tolerance in said stably transformed plant, plant part, and/or plant cell as compared to a control, wherein the heterologous polynucleotide encoding a ferredoxin polypeptide and the heterologous polynucleotide encoding a biotin ligase polypeptide are from a bacterial and/or an archaeal species.

30 In some aspects, the methods of the invention further comprise, consist essentially of, or consist of regenerating a stably transformed plant or plant part from the stably transformed plant cell, wherein expression of one or more of the heterologous polynucleotides in said regenerated and stably transformed plant or plant part results in the stably transformed plant and/or plant part having increased carbon fixation and/or increased biomass production

and/or increased abiotic stress tolerance as compared to a control (e.g., a plant or plant part not transformed with and stably expressing said heterologous polynucleotides).

Thus, in some embodiments, a method for producing a plant having increased carbon fixation and/or increased biomass production is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased carbon fixation and/or increased biomass production as compared to a control (e.g., a plant not comprising said heterologous polynucleotide), wherein the heterologous polynucleotide encoding a ferredoxin polypeptide is from a bacterial and/or an archaeal species.

In other embodiments, a method for producing a plant having increased carbon fixation and/or increased biomass production is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased carbon fixation and/or increased biomass production as compared to a control, wherein said heterologous polynucleotide encoding a biotin ligase polypeptide is from a bacterial and/or an archaeal species.

In further embodiments, a method for producing a plant having increased carbon fixation and/or increased biomass production is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased carbon fixation and/or increased biomass production as compared to a control, wherein said heterologous polynucleotide encoding a ferredoxin polypeptide and said heterologous polynucleotide encoding a biotin ligase polypeptide are from a bacterial and/or an archaeal species.

In additional embodiments, a method for producing a plant having increased abiotic stress tolerance is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased abiotic stress tolerance as compared to a control (e.g., a plant, plant part, and/or plant cell not comprising said heterologous polynucleotide), wherein the heterologous polynucleotide encoding a ferredoxin polypeptide is from a bacterial and/or an archaeal species.

In other embodiments, a method for producing a plant having increased abiotic stress tolerance is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased abiotic stress tolerance as compared to a control, wherein said heterologous polynucleotide encoding a biotin ligase polypeptide is from a bacterial and/or an archaeal species.

In further embodiments, a method for producing a plant having increased abiotic stress tolerance is provided, comprising, consisting essentially of, or consisting of: introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide, thereby producing a stably transformed plant, plant part, and/or plant cell having increased abiotic stress tolerance as compared to a control, wherein said heterologous polynucleotide encoding a ferredoxin polypeptide and said heterologous polynucleotide encoding a biotin ligase polypeptide are from a bacterial and/or an archaeal species.

The polypeptides ferredoxin and biotin ligase, and the polynucleotides that encode said polypeptides are known in the art and are produced by many different organisms. In some embodiments, a ferredoxin polypeptide (e.g., a polypeptide having the activity of a ferredoxin polypeptide) and biotin ligase polypeptide (e.g., a polypeptide having the activity of a biotin ligase polypeptide) useful with this invention can be any archaeal polypeptide or bacterial polypeptide useful for biological carbon sequestration and increasing abiotic stress tolerance and having the enzyme activity of ferredoxin or biotin ligase. Examples of organisms from which these polypeptides and polynucleotides can be derived include, but are not limited to, *Escherichia coli*, *Rhodopseudomonas palustris*, *Methanosarcina acetivorans*, *Haloarcula japonica*, *Pyrococcus furiosus*, *Hydrogenobacter thermophilus*, *Clostridium ljungdahlii*, *Rhizobium etli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Deinococcus radiodurans*, and/or *Methanosarcina barkeri*.

In some embodiments, a ferredoxin polypeptide and/or polynucleotide encoding a ferredoxin can be from *Escherichia coli*, *Rhodopseudomonas palustris*, *Methanosarcina acetivorans*, *Haloarcula japonica*, *Pyrococcus furiosus*, *Hydrogenobacter thermophilus*, *Clostridium ljungdahlii*, or any combination thereof.

In some embodiments, a biotin ligase polypeptide and/or polynucleotide encoding a biotin ligase can be from *Escherichia coli*, *Rhizobium etli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Deinococcus radiodurans*, *Methanosarcina barkeri*, or any combination thereof.

More particularly, a polynucleotide encoding a ferredoxin polypeptide useful with this invention can include, but is not limited to, a polynucleotide from *Hydrogenobacter thermophilus* TK-6 (see, e.g., **SEQ ID NO:1**), from *Clostridium ljungdahlii* (see, e.g., **SEQ ID NO:3**) *Escherichia coli* (see, e.g., **SEQ ID NO:5**), *Rhodopseudomonas palustris* (see, e.g., **SEQ ID NO:7**), *Methanosarcina acetivorans* (see, e.g., **SEQ ID NO:9**), *Haloarcu-
5 japonica* (see, e.g., **SEQ ID NO:11**), and/or *Pyrococcus furiosus* (see, e.g., **SEQ ID NO:13**, **SEQ ID NO:15**). In other embodiments, a ferredoxin polypeptide useful with this invention includes, but is not limited to, a ferredoxin polypeptide having an amino acid sequence that includes, but is not limited to, an amino acid sequence from *Hydrogenobacter thermophilus*
10 TK-6 (see, e.g., **SEQ ID NO:2**), *Clostridium ljungdahlii* (see, e.g., **SEQ ID NO:4**), *Escherichia coli* (see, e.g., **SEQ ID NO:6**), *Rhodopseudomonas palustris* (see, e.g., **SEQ ID NO:8**), *Methanosarcina acetivorans* (see, e.g., **SEQ ID NO:10**), *Haloarcu-
japonica* (see, e.g., **SEQ ID NO:12**), and/or *Pyrococcus furiosus* (see, e.g., **SEQ ID NO:14**, **SEQ ID NO:16**). In some particular embodiments, a heterologous polynucleotide encoding a
15 ferredoxin polypeptide comprises, consists essentially of, or consists of the nucleotide sequence of **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:3**, the nucleotide sequence of **SEQ ID NO:5**, the nucleotide sequence of **SEQ ID NO:7**, the nucleotide sequence of **SEQ ID NO:9**, the nucleotide sequence of **SEQ ID NO:11**, the nucleotide sequence of **SEQ ID NO:13**, and/or the nucleotide sequence of **SEQ ID NO:15**.

In some embodiments, a polynucleotide encoding a biotin ligase polypeptide useful with this invention includes, but is not limited to, a polynucleotide from *Escherichia coli* (see, e.g., **SEQ ID NO:17**), *Rhizobium etli* (see, e.g., **SEQ ID NO: 19**), *Bacillus subtilis* (see, e.g., **SEQ ID NO: 21**), *Corynebacterium glutamicum* (see, e.g., **SEQ ID NO: 23**), *Deinococcus radiodurans* (see, e.g., **SEQ ID NO: 25**), and/or *Methanosarcina barkeri* (see,
25 e.g., **SEQ ID NO: 27**). In some embodiments, a biotin ligase polypeptide useful with this invention includes, but is not limited to, an amino acid sequence from *Escherichia coli* (see, e.g., **SEQ ID NO: 18**), *Rhizobium etli* (see, e.g., **SEQ ID NO: 20**), *Bacillus subtilis* (see, e.g., **SEQ ID NO: 22**), *Corynebacterium glutamicum* (see, e.g., **SEQ ID NO: 24**), *Deinococcus radiodurans* (see, e.g., **SEQ ID NO: 26**), and/or *Methanosarcina barkeri* (see, e.g., **SEQ ID**
30 **NO: 28**). Thus, in some particular embodiments, a heterologous polynucleotide encoding a biotin ligase polypeptide comprises, consists essentially of, or consists of the nucleotide sequence of **SEQ ID NO:17**, the nucleotide sequence of **SEQ ID NO:19**, the nucleotide sequence of **SEQ ID NO:21**, the nucleotide sequence of **SEQ ID NO:23**, the nucleotide sequence of **SEQ ID NO:25**, and/or the nucleotide sequence of **SEQ ID NO:27**.

In some embodiments, a heterologous polynucleotide encoding a biotin ligase polypeptide can be from *E. coli* strain AVB101/K-12. In representative embodiments, a heterologous polynucleotide encoding a biotin ligase polypeptide from *E. coli* strain K AVB101/K-12 comprises, consists essentially of, or consists of the nucleotide sequence of **SEQ ID NO:17** (*BirA*). In some embodiments, a heterologous polynucleotide encoding a ferredoxin polypeptide can be from *Hydrogenobacter thermophilus* TK-6. In representative embodiments, a heterologous polynucleotide encoding a ferredoxin polypeptide from *Hydrogenobacter thermophilus* TK-6 comprises, consists essentially of, or consists of the nucleotide sequence of **SEQ ID NO:1**.

In further embodiments, polypeptides and the polynucleotides encoding said polypeptides can be modified for use with this invention. For example, a polypeptide modification useful with this invention include amino acid substitutions (and the corresponding base pair changes in the respective polynucleotide encoding said polypeptide). Thus, in some embodiments, a polypeptide and/or polynucleotide sequence of the invention can be a conservatively modified variant. As used herein, "conservatively modified variant" refers to polypeptide and polynucleotide sequences containing individual substitutions, deletions or additions that alter, add or delete a single amino acid or nucleotide or a small percentage of amino acids or nucleotides in the sequence, where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

As used herein, a conservatively modified variant of a polypeptide is biologically active and therefore possesses the desired activity of the reference polypeptide (e.g., ferredoxin, biotin ligase) as described herein. The variant can result from, for example, a genetic polymorphism or human manipulation. A biologically active variant of the reference polypeptide can have at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity (e.g., about 30% to about 99% or more sequence identity and any range therein) to the amino acid sequence for the reference polypeptide as determined by sequence alignment programs and parameters described elsewhere herein. An active variant can differ from the reference polypeptide sequence by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

Naturally occurring variants may exist within a population. Such variants can be identified by using well-known molecular biology techniques, such as the polymerase chain reaction (PCR), and hybridization as described below. Synthetically derived nucleotide

sequences, for example, sequences generated by site-directed mutagenesis or PCR-mediated mutagenesis which still encode a polypeptide of the invention, are also included as variants. One or more nucleotide or amino acid substitutions, additions, or deletions can be introduced into a nucleotide or amino acid sequence disclosed herein, such that the substitutions, additions, or deletions are introduced into the encoded protein. The additions (insertions) or deletions (truncations) may be made at the N-terminal or C-terminal end of the native protein, or at one or more sites in the native protein. Similarly, a substitution of one or more nucleotides or amino acids may be made at one or more sites in the native protein.

For example, conservative amino acid substitutions may be made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue with a similar side chain. Families of amino acid residues having similar side chains are known in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity.

In some embodiments, amino acid changes can be made to alter the catalytic activity of an enzyme. For example, amino acid substitutions can be made to a thermoactive enzyme that has little activity at room temperature (*e.g.*, about 20°C to about 50°C) so as to increase activity at these temperatures. A comparison can be made between the thermoactive enzyme and a mesophilic homologue having activity at the desired temperatures. This can provide discrete differences in amino acids that can then be the focus of amino acid substitutions.

Thus, in some embodiments, amino acid sequence variants of a reference polypeptide can be prepared by mutating the nucleotide sequence encoding the enzyme. The resulting mutants can be expressed recombinantly in plants, and screened for those that retain biological activity by assaying for the enzyme activity (*e.g.*, ferredoxin activity, biotin ligase activity) using standard assay techniques as described herein. Methods for mutagenesis and

nucleotide sequence alterations are known in the art. See, e.g., Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods in Enzymol.* 154:367-382; and *Techniques in Molecular Biology* (Walker & Gaastra eds., MacMillan Publishing Co. 1983) and the references cited therein; as well as US Patent No. 4,873,192. Clearly, the mutations made in the DNA encoding the variant must not disrupt the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, D.C.).

The deletions, insertions and substitutions in the polypeptides described herein are not expected to produce radical changes in the characteristics of the polypeptide (e.g., the temperature at which the polypeptide is active). However, when it is difficult to predict the exact effect of the substitution, deletion or insertion in advance of doing so, one of skill in the art will appreciate that the effect can be evaluated by routine screening assays for the particular polypeptide activities of interest (e.g., ferredoxin activity, biotin ligase activity) as described herein.

In some embodiments, the compositions of the invention can comprise functional fragments of the polypeptide. As used herein, "functional fragment" means a portion of the reference polypeptide that retains the polypeptide activity of, for example, ferredoxin, or biotin ligase. A fragment also means a portion of a nucleic acid molecule encoding the reference polypeptide. An active fragment of the polypeptide can be prepared, for example, by isolating a portion of a polypeptide-encoding nucleic acid molecule that expresses an encoded fragment of the polypeptide (e.g., by recombinant expression *in vitro*), and assessing the activity of the fragment. Nucleic acid molecules encoding such fragments can be at least about 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, or 1,500 contiguous nucleotides, or up to the number of nucleotides present in a full-length polypeptide-encoding nucleic acid molecule. As such, polypeptide fragments can be at least about 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 contiguous amino acid residues, or up to the total number of amino acid residues present in the full-length polypeptide.

Methods for assaying the activities of a ferredoxin polypeptide and/or a biotin ligase polypeptide are known in the art. Exemplary activity assays for these enzymes are set forth below.

Ferredoxin. Ferredoxins assayed indirectly using ferredoxin dependent reduction of oxidoreductases such as 2-oxoacid:ferredoxin oxidoreductase. An exemplary assay includes a standard 2-oxoacid:ferredoxin oxidoreductase activity assay. These assays are carried out at the temperature that is appropriate for the selected enzyme, for example, 55°C, the temperature for an oxidoreductase enzyme from a thermophile. Changes in absorbance are monitored at 550 nm (due to the ferredoxin dependent reduction of a horse heart cytochrome c in the presence of 2-oxoglutarate as a substrate) using a modified version of the method described by Kerscher et al. (Kerscher, et al. (1982) *Eur. J. Biochem.* 128, 223-230; see also, Iwasaki, T. and Oshima, T. (2001) Ferredoxin and related enzymes from *Sulfolobus*; in *Methods in Enzymology* 334, Adams, M. W. W. (ed.), pp. 3-22, Academic Press, New York, USA.). Briefly, the enzyme reaction is started by adding 0.1 ml of the purified enzyme solution (50 µg/ml) to 0.1 ml of pre-warmed purified ferredoxin solution (260 µg/ml) and 0.8 ml of pre-warmed 100 mM sodium phosphate buffer (pH 7.0) containing 20 µM horse heart cytochrome c, 20 µM coenzyme A, and 4 mM 2-oxoglutarate. For all measurement, the effect of cytochrome c nonenzymatic reduction is taken into consideration and subtracted from values determined in the presence of enzyme. Measurements are carried out at least three times. Standard deviations never exceed 10% of the mean values. One enzyme unit is defined as the amount of enzyme required to reduce 1 µmol of cytochrome c per min at 55°C. A reduction of cytochrome c by 1 nmol corresponds to an A_{550nm} increase of 0.021 (Kerscher et al., 1982).

Biotin ligase. In an exemplary biotin ligase assay, biotin ligase mediated biotinylation of a target peptide is detected by its interaction with streptavidin-conjugated alkaline phosphatase. Briefly, about 100 µg of target peptide is incubated for about 5 h at about 30°C with biotin ligase in 50 mM bicine buffer (pH 8.3) containing 10 mM ATP, 10 mM MgCl₂ and 50 mM biotin. The reaction mixture is then desalted on a PD10 column (Amersham, Little Chalfont, UK) and concentrated by ultrafiltration. Detection of the biotinylation by its interaction with streptavidin-conjugated alkaline phosphatase can be done as described in Czerwinski et al. (*New Biotechnology* 26(5):215-221 (2009)) or as described in the Avidity L.L.C. biotinylation reaction assay (www.avidity.com/sites/default/files/protocol-pdf/BRTA%20protocol.pdf).

These assays can be performed on protein extracts from plants, plant parts (e.g., leaf, stem, seed, and the like) and plant cells (e.g., cell cultures comprising tissue culture, a suspension of plant cells such as algal cells, protoplasts and the like).

In some embodiments, a heterologous polynucleotide encoding a ferredoxin

polypeptide and/or a biotin ligase polypeptide as well as any other heterologous polynucleotide encoding a polypeptide or functional nucleic acid of interest can be comprised within an expression cassette. As used herein, "expression cassette" means a recombinant nucleic acid molecule comprising at least one polynucleotide sequence of interest (e.g., a
5 heterologous polynucleotide encoding a biological carbon sequestration polypeptide, and the like), wherein said recombinant nucleic acid molecule is operably associated with at least one control sequence (e.g., a promoter). Thus, some embodiments of the invention provide expression cassettes designed to express a recombinant nucleic acid molecule/heterologous polynucleotide encoding polypeptides having the enzyme activity of ferredoxin and/or biotin
10 ligase.

An expression cassette comprising a recombinant nucleic acid molecule 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 also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

15 In some embodiments, where the ferredoxin and/or a biotin ligase are both to be introduced, the heterologous polynucleotides encoding a ferredoxin and/or a biotin ligase can be comprised in a single expression cassette. In some embodiments, the single expression cassette can further comprise a heterologous polynucleotide encoding any other polypeptide or functional nucleic acid of interest. The expression cassette can be operably linked to a
20 promoter that drives expression of all of the polynucleotides comprised in the expression cassette and/or the expression cassette can comprise one or more promoters operably linked to one or more of the heterologous polynucleotides for driving the expression of said heterologous polynucleotides individually or in combination. In other embodiments, the heterologous polynucleotides encoding a ferredoxin polypeptide and/or a biotin ligase
25 polypeptide (and/or any other polypeptide or functional nucleic acid of interest) can be comprised in separate expression cassettes.

When the heterologous polynucleotides are comprised within more than one expression cassette, said heterologous polynucleotides encoding ferredoxin and/or biotin ligase can be introduced into plants singly or more than one at a time using co-transformation
30 methods as known in the art. In addition to transformation technology, traditional breeding methods as known in the art can be used to assist in introducing into a single plant (when so desired) each of the polynucleotides encoding the ferredoxin and/or biotin ligase enzymes, alone or in combination as described herein, and/or any additional polynucleotides of interest

to produce a plant, plant part, and/or plant cell comprising and expressing ferredoxin and/or biotin ligase and/or any additional desired heterologous polynucleotides of interest.

Any promoter useful for initiation of transcription in a cell of a plant 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 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 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).

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 the polypeptides of the crTCA cycle as described herein 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), or plant cells (including algae 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 to be transformed and then inserted into the expression cassette to be used in transformation of the plant.

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.

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), *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 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 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) and U.S. Patent 5,614,395. In some embodiments, a promoter for chemical induction can be the tobacco PR-1a promoter.

In 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 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 (Prn), 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 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)).

In some embodiments, the heterologous polynucleotides of the invention (e.g., the ferredoxin and biotin ligase polypeptides described herein) can be transformed into the nucleus or into, for example, the chloroplast using standard techniques known in the art of plant transformation.

Thus, in some embodiments, a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide can be transformed into and expressed in the nucleus and the polypeptides produced remain in the cytosol. In other embodiments, a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide can be transformed into and expressed in the nucleus, wherein one or more of the polypeptides can be targeted to the chloroplast. Thus, in particular embodiments, a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide can be operably associated with at least one targeting nucleotide sequence encoding a signal peptide that targets the polypeptides to the chloroplast.

A signal sequence may be operably linked at the N- or C- terminus of a heterologous nucleotide sequence or nucleic acid molecule. 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 1**.

Table 1. Amino acid sequences of representative signal peptides.

Source	Sequence	Target
Rubisco small subunit (tobacco)	MASSVLSSAAVATRSNVAQANMVAPFTGLKSAASFPVSR KQNLDTISIASNGGRVQC (SEQ ID NO:29)	chloroplast
Arabidopsis proline-rich protein 2 (AT2G21140)	MRILPKSGGGALCLLFVFALCSVAHS (SEQ ID NO:30)	cell wall/secretory pathway
PTS-2 (conserved in eukaryotes)	RLX ₅ HL (SEQ ID NO:31) MRLSIHAEHL (SEQ ID NO:32) SKL	peroxisome
Arabidopsis presequence protease1 (AT3G19170)	MLRTVSCLASRSSSSSLFFRFRQFPRSYSMLTSSTAALRVPSRNLR RISSPSVAGRLLLRRLRIPSAAVRSVNGQFSRLSVRA (SEQ ID NO:33)	mitochondria and chloroplast
<i>Chlamydomonas reinhardtii</i> -(Stroma-targeting cTPs:	MALVARPVLSARVAASRPRVAARKAVRVSAKYGEN (SEQ ID NO:34)	chloroplast

photosystem I (PSI) subunits P28, P30, P35 and P37, respectively)	MQALSSRVNIAAKPQRAQLVVRAEEVKA (SEQ ID NO:35) MQTLASRPSLRASARVAPRRAPRVAVVTKAALDPQ (SEQ ID NO:36) MQALATRPSAIRPTKAARRSSVVVRADGFIG (SEQ ID NO:37)	
<i>C. reinhardtii</i> – chlorophyll a/b protein (cabII-1)	MAFALASRKALQVTCKATGKKTAAKAAAPKSSGVEFYGPNAK WLGPYSEN (SEQ ID NO:38)	chloroplast
<i>C. reinhardtii</i> – Rubisco small subunit	MAAVIAKSSVSAAVARPARSSVRPMAALKPAVKAAPVAAPAQA NQMMVWT (SEQ ID NO:39)	chloroplast
<i>C. reinhardtii</i> – ATPase-γ	MAAMLASKQGAFAFMGRSSFAPAPKGVASRGSLLQVVAGLKEV (SEQ ID NO:40)	chloroplast
<i>Arabidopsis thaliana</i> abscisic acid receptor PYL10	CVVQ (SEQ ID NO:41)	membrane
<i>Saccharomyces cerevisiae</i> cox4	MLSLRQSIRFFKPATRTLCSRYLL (SEQ ID NO:46)	mitochondria
<i>Arabidopsis</i> aconitase	MYLTASSSASSSIIRAASSRSSSLFSFRSVLSPSVSSTSPSSLL ARRSFGTISPAFRRWSHSFHSKPSPFRFTSQIRA (SEQ ID NO:47)	mitochondria
Yeast aconitase	MLSARSAIKRPIVRGLATV (SEQ ID NO:48)	mitochondria

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: 42).

Thus, in representative embodiments of the invention, a heterologous polynucleotide encoding ferredoxin and/or a heterologous polynucleotide encoding biotin ligase 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:29, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, or SEQ ID NO:39.

In other embodiments of the invention, a heterologous polynucleotide encoding ferredoxin and/or a heterologous polynucleotide encoding biotin ligase to be expressed in a plant, plant part or plant cell can be operably linked to a mitochondrial targeting sequence encoding a mitochondrial signal peptide, optionally wherein said mitochondrial signal peptide is encoded by an amino acid sequence that includes, but is not limited to, the amino acid sequence of SEQ ID NO:46, SEQ ID NO:47, or SEQ ID NO:48.

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 plasma membrane 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 (**SEQ ID NO:41**). The addition of prenyl groups
10 facilitates membrane association and protein-protein interactions of the prenylated proteins.

In still other embodiments of the invention, a signal peptide can direct a polypeptide of the invention to more than one organelle (e.g., dual targeting). Thus, in some embodiments, a signal peptide that can target a polypeptide of the invention 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:33**.

In addition to promoters operably linked to a heterologous polynucleotide of the invention, 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
20 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
25 include at least one intron. An intron useful with this invention can be an intron identified in and isolated from a plant to be transformed and then inserted into the expression cassette to be used in transformation of the plant. 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
30 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. 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 be native to the
15 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
20 *rbcS* E9 terminator, a RubisCo small subunit gene 1 (*TrbcS1*) terminator, an actin gene (*Tactin*) terminator, a nitrate reductase gene (*Tnr*) terminator, and/or aa duplicated carbonic anhydrase gene 1 (*Tdca1*) terminator.

Further non-limiting examples of terminators useful with this invention for expression of the heterologous polynucleotides of the invention or other heterologous polynucleotides of
25 interest in algae include a terminator of the *psbA* gene (*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*).

30 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 and/or plant cell. As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to a plant, plant part and/or plant cell expressing the marker and thus allows such a transformed plant, plant part, and/or plant 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
 5 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.*,
 10 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
 15 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
 20 (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.
 25 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
 30 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. 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 of the invention (e.g., polynucleotide(s) of the invention encoding biological carbon sequestration polypeptides and/or conferring increased abiotic stress tolerance (e.g., ferredoxin and/or biotin ligase)), also can optionally include additional polynucleotides that encode other desired traits. Such desired traits can be, for example, polynucleotides which confer high light tolerance, increased drought tolerance, increased flooding tolerance, increased tolerance to soil contaminants, 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, 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 and/or plant cells having the desired 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 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*). 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.*, International Patent Application Publication Nos. WO 99/25821; WO 99/25854; WO 99/25840; WO 99/25855 and WO 99/25853.

Any nucleotide sequence to be transformed into a plant, plant part and/or plant 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 polynucleotide sequences. In those embodiments in which each of codons in native polynucleotide 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 polynucleotide sequence, site-directed mutagenesis can be used according to methods known in the art (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. Thus, in some embodiments, the heterologous polynucleotides encoding ferredoxin and/ or biotin ligase can be codon optimized for expression in the particular organism into which the polynucleotides are to be expressed.

A heterologous polynucleotide encoding a ferredoxin polypeptide and/or a biotin

ligase polypeptide as described herein; and/or functional fragments thereof (e.g., a functional fragment of the nucleotide sequences of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and/or any combination thereof or the amino acid sequences of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and/or any combination thereof) can be introduced
5 into a cell of a plant 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 are well known and routine in the art and are
10 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
15 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
20 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)).

A polynucleotide therefore can be introduced into a plant, plant part, plant cell in any number of ways that are well known in the art. The methods of the invention do not depend
25 on a particular method for introducing one or more nucleotide sequences into a plant, only that they gain access to the interior the cell. Where more than 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
30 transformation event, or in separate transformation events, or, alternatively, a polynucleotide can be incorporated into a plant as part of a 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 a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin

ligase polypeptide as described herein. Means for regeneration can vary from plant species to plant species, but generally a suspension of transformed protoplasts or a petri 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 may also be advantageous to add glutamic acid and 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.

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 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.

Accordingly, in some aspects of the invention, a stably transformed plant, plant part and/or plant cell is provided, which comprises in its genome one or more recombinant nucleic acid molecules/heterologous polynucleotides of the invention (e.g., heterologous polynucleotides encoding ferredoxin and/or biotin ligase) and has increased carbon fixation and/or increased biomass production and/or increased abiotic stress tolerance when compared to a control (e.g., a plant not comprising in its genome the one or more recombinant nucleic acid molecules/heterologous polynucleotides of the invention). Thus, in some embodiments, the invention provides a stably transformed plant, plant part and/or plant cell comprising in

its genome at least one heterologous polynucleotide encoding a ferredoxin polypeptide and/or a biotin ligase polypeptide, which when expressed results in the stably transformed plant, plant part or plant cell having increased carbon fixation and/or increased biomass production and/or increased abiotic tolerance as compared to a control. In representative embodiments, a
5 heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide, when expressed in a plant, may be expressed in the nucleus and targeted to the chloroplast and/or may be expressed in the chloroplast.

Additionally provided herein are seeds produced from the stably transformed plants of
10 the invention, wherein said seeds comprise in their genome a heterologous polynucleotide encoding a ferredoxin polypeptide and/or a heterologous polynucleotide encoding a biotin ligase polypeptide, wherein said heterologous polynucleotides are from a bacterial or archaeal species.

The present invention further provides products produced from the stably transformed
15 plant, plant cell or plant part of the invention. In some embodiments, the product produced can include but is not limited to biofuel, food, drink, animal feed, fiber, and/or pharmaceuticals.

The following examples are not intended to be a detailed catalog of all the different
20 ways in which the present invention may be implemented or of all the features that may be 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. Enhancing biological carbon sequestration in a plant by expression of heterologous ferredoxin and biotin ligase.

A report (Jitrapakdee et al. *Biochemical Journal*, 413:369-387 (2010)) describing the structure of pyruvate carboxylase, an enzyme that is highly structurally related to OGC,
30 demonstrates that the carboxylase enzyme is charged with biotin (see, Fig. 4 of Jitrapakdee et al.). Biotin is important because it holds the CO group before it is placed onto the substrate. Without biotin, there is no carbon fixation. The carboxylase becomes charged with biotin in a post-translational modification, which is carried out by another protein known as Biotin Protein Ligase (*E. coli*) or Holocarboxylase Synthetase (*Arabidopsis*). Amino acid sequence

alignments, as shown in **Fig. 1**, indicate that these two proteins share little sequence similarity, suggesting that the plant biotin ligase may not be able to properly biotinylate a bacterial enzyme.

In addition to the difference in the amino acid sequences for BirA and

5 Holocarboxylase synthetase, the synthetase activity in the chloroplast may be too low to support biotinylation of the OGC. Tissot et al. (*European Journal of Biochemistry*, 258(2):586-596 (1998)) reported that even though they detected Holocarboxylase Synthetase activity in the chloroplast of pea leaves, the activity was low. Besides the Biotin Protein Ligase which will support OGC activity, we believe that the KOR enzyme may also benefit
10 from the addition of a compatible ferredoxin. The KOR enzyme requires reduction by a compatible ferredoxin in order to produce 2-oxoglutarate. It has been shown that the HyTh-KOR is specific for the HyTh-Fdx and shows no activity with the 2Fe-2S type ferredoxin from *Chlorella* spp. or a 4Fe-4S type ferredoxin from *Clostridium pasteurianum* (Yoon et al. *Journal of Bacteriology*, 178(11):3365-3368 (1996). However, the authors do note that the
15 thermostability of these ferredoxins may have played a role in their inability to interact with the HyTh-KOR. There are 4Fe-4S type ferredoxins in plant mitochondria, but the ferredoxins in the chloroplast are all 2Fe-2S type. While this does not implicitly rule out that the HyTh-KOR may be able to exchange electrons with the plant ferredoxins in the chloroplast, it does generate doubt as to whether or not this reaction will be able to perform efficiently without its
20 preferred ferredoxin. We have done LC-MS ¹³C exchange experiments to assess the ability of the HyTh-KOR to exchange electrons with a spinach 2Fe-2S type ferredoxin purchased from Sigma and demonstrated that the spinach ferredoxin could support HyTh KOR function but that HyTh ferredoxin did enable somewhat higher concentrations of 2-oxoglutarate to be produced by HyTh KOR, which suggests that provision of the HyTh Fdx *in planta* could
25 improve *in planta* HyTh KOR function.

To express a bacterial biotin ligase and ferredoxin *in planta*, Construct #3 was generated, which consists of an *E. coli* BirA sequence (Construct_3_BirA (**SEQ ID NO:43**)) and *Hydrogenobacter thermophilus* ferredoxin sequence (Construct_3_HyThFDX (**SEQ ID NO:44**)). These constructs were optimized for expression in Camelina and synthesized for
30 cloning into pCAMBIA_2300_mch/BASTA cloned into p_CAMBIA_2300 (see **Fig. 2**). The sequence of Construct #3 was verified (see, **SEQ ID NO:45** for the entire Construct #3 sequence) and used to transform camelina.

The growth of wild type and T1 generation Construct #3 transformed camelina plants was assessed at 21 days after planting (DAP) by measuring the height of the plants. It was

observed at 21 DAP that the height of Construct #3 transformed plants was double that of the wild type plants (18 cm for the C3 transformed plants compared to 9 cm for wild type, see Fig. 3).

5 **Example 2. Enhancing electron transfer to reduce oxidative stress.**

Under many environmental conditions electron transport can exceed ATP synthesis in the chloroplast and generate oxidative stress. Increasing the pool of ferredoxin by expression of a bacterial or archaeal ferredoxin, such as that from *Escherichia coli*, *Rhodospseudomonas palustris*, *Methanosarcina acetivorans*, *Haloarcula japonica*, *Pyrococcus furiosus*,
 10 *Hydrogenobacter thermophilus*, and/or *Clostridium ljungdahlii* in the chloroplast might enable an additional electron shunt to reduce the electron pressure at photosystem I and cyclic electron transport which would lead to further increase in the proton motive force across the thylakoid membrane. Expression of bacterial or archaeal ferredoxin may reduce oxidative stress.

15 Plants, such as camelina, are transformed using constructs comprising bacterial or archaeal ferredoxin such as, for example, *Hydrogenobacter thermophilus* ferredoxin, as described herein, thereby reducing oxidative stress and increasing biomass production and abiotic stress tolerance in the transgenic plants.

20 **Example 3. Enhancing CO₂ assimilation and fatty acid production by expression of a bacterial Biotin Protein Ligase in plants by improving stability of endogenous biotin-containing carboxylases.**

Many plant carboxylases contain biotin as a co-factor (Wurtele et al. *Archives of biochemistry and biophysics* 278 (1990): 179-186). Biogenesis of these enzymes is dependent
 25 on the availability of biotin and the assembly into the holoenzyme. It has been shown that plant biotin ligase (Holocarboxylase synthetases; HCSs) can be functionally active in bacteria and complement bacterial biotin ligase mutants (Tissot et al. *European journal of biochemistry* 258 (1998): 586-596). Expression of heterologous Biotin-ligases may accelerate the biogenesis and stability of endogenous carboxylases in plants. Carboxylases are present in
 30 all cellular compartments and central to many key metabolic pathways like lipid and amino acid metabolism.

Plants, such as camelina, are transformed using constructs comprising bacterial or archaeal biotin ligase, such as that from *Escherichia coli*, *Rhizobium etli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Deinococcus radiodurans*, and/or *Methanosarcina barkeri*, as

described herein, thereby increasing biomass production and abiotic stress tolerance in the transgenic plants.

The above examples clearly illustrate the advantages of the invention. Although the present invention has been described with reference to specific details of certain
5 embodiments thereof, it is not intended that such details should be regarded as limitations 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 method for increasing carbon fixation and/or increasing biomass production in a plant, comprising:

5 introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby increasing the carbon fixation and/or increasing biomass production as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

10 2. A method for increasing carbon fixation and/or increasing biomass production in a plant, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or
15 plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby increasing the carbon fixation and/or increasing biomass production as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

3. A method for increasing abiotic stress tolerance in a plant, comprising:

20 introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby increasing the abiotic stress tolerance as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

25 4. A method for increasing abiotic stress tolerance in a plant, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or
30 plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby increasing the abiotic stress tolerance as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

5. A method for producing a plant having increased carbon fixation, increasing biomass and/or production, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby producing a plant having increased carbon fixation and/or increasing biomass production as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

6. A method for producing a plant having increased carbon fixation and/or increasing biomass production, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby producing a plant having increased carbon fixation and/or increasing biomass production as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

7. A method for producing a plant having increased abiotic stress tolerance, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a ferredoxin polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein the heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby producing a plant having increased abiotic stress tolerance as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

8. A method for producing a plant having increased abiotic stress tolerance, comprising:

introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide to produce a stably transformed plant, plant part, and/or plant cell, wherein said heterologous polynucleotide is from a bacterial and/or an archaeal species, thereby producing a plant having increased abiotic stress tolerance as compared to a plant, plant part, or plant cell not comprising said heterologous polynucleotide.

9. The method of any one of claims 1, 3, 5 or 7 comprising introducing into a plant, plant part, and/or plant cell a heterologous polynucleotide encoding a biotin ligase polypeptide.

10. The method of any one of claims 1, 3, 5 or 7, wherein the heterologous polynucleotide encoding a ferredoxin polypeptide is operably linked to a promoter.

11. The method of any one of claims 2, 4, 6, 8 or 9, wherein the heterologous
5 polynucleotide encoding a biotin ligase polypeptide is operably linked to a promoter.

12. The method of any one of claims 1 to 11, wherein the heterologous polynucleotide encoding a ferredoxin polypeptide and/or the heterologous polynucleotide encoding a biotin
ligase polypeptide, are introduced into a nucleus and/or a chloroplast of said plant, plant part,
10 and/or plant cell.

13. The method of any one of claims 1 to 12, wherein the heterologous polynucleotide encoding a ferredoxin polypeptide and/or the heterologous polynucleotide encoding a biotin
ligase polypeptide, are introduced into a nucleus of said plant, plant part, and/or plant cell and
15 are operably linked to an amino acid sequence that targets said polypeptides to the
chloroplast.

14. The method of any one of claims 1, 3, 5, 7, 10, 12 or 13, wherein the heterologous
polynucleotide encoding the ferredoxin polypeptide is from *Escherichia coli*,
20 *Rhodopseudomonas palustris*, *Methanosarcina acetivorans*, *Haloarcula japonica*,
Pyrococcus furiosus, *Hydrogenobacter thermophilus*, *Clostridium ljungdahlii*, or any
combination thereof.

15. The method of any one of claims 2, 4, 6, 8, 9, or 11 to 14 wherein the heterologous
25 polynucleotide encoding the biotin ligase polypeptide is from *Escherichia coli*, *Rhizobium*
etli, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Deinococcus radiodurans*,
Methanosarcina barkeri, or any combination thereof.

16. A stably transformed plant, plant part or plant cell produced by the method of any of
30 the claims 5 to 15.

17. A stably transformed plant, plant part or plant cell comprising a heterologous
polynucleotide encoding a ferredoxin polypeptide, wherein said heterologous polynucleotide
encoding a ferredoxin polypeptide is from a bacterial and/or an archaeal species.

18. A stably transformed plant, plant part or plant cell comprising a heterologous polynucleotide encoding a biotin ligase polypeptide, wherein said heterologous polynucleotide encoding a biotin ligase polypeptide is from a bacterial and/or an archaeal species.
- 5 19. A stably transformed plant, plant part or plant cell comprising a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide, wherein said heterologous polynucleotide encoding a ferredoxin polypeptide and said heterologous polynucleotide encoding a biotin ligase
- 10 polypeptide are from a bacterial and/or an archaeal species.
20. The stably transformed plant of any one of claims 17 or 19, wherein the heterologous polynucleotide encoding the ferredoxin polypeptide is from *Escherichia coli*, *Rhodopseudomonas palustris*, *Methanosarcina acetivorans*, *Haloarcula japonica*,
- 15 *Pyrococcus furiosus*, *Hydrogenobacter thermophilus*, *Clostridium ljungdahlii*, or any combination thereof.
21. The stably transformed plant of any one of claims 18 or 19 wherein the heterologous polynucleotide encoding the biotin ligase polypeptide is from *Escherichia coli*, *Rhizobium*
- 20 *etli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Deinococcus radiodurans*, *Methanosarcina barkeri*, or any combination thereof.
22. A seed of the stably transformed plant of claim 16, wherein the seed comprises in its genome a heterologous polynucleotide encoding a ferredoxin polypeptide (from a bacterial or
- 25 an archaeal species) and/or a biotin ligase polypeptide (from a bacterial or an archaeal species).
23. A seed of the stably transformed plant of claim 17, wherein the seed comprises in its genome a heterologous polynucleotide encoding a ferredoxin polypeptide (from a bacterial or
- 30 an archaeal species).
24. A seed of the stably transformed plant of claim 18, wherein the seed comprises in its genome a heterologous polynucleotide encoding a biotin ligase polypeptide from a bacterial or an archaeal species.

25. A seed of the stably transformed plant of claim 19, wherein the seed comprises in its genome a heterologous polynucleotide encoding a ferredoxin polypeptide from a bacterial or an archaeal species and a heterologous polynucleotide encoding a biotin ligase polypeptide
5 from a bacterial or an archaeal species.
26. A product produced from the stably transformed plant, plant part or plant cell of any one of claims 16 to 21, or the stably transformed seed of any one of claims 22 to 25.
- 10 27. The product of claim 26, wherein the product is a food, drink, animal feed, fiber, oil, pharmaceutical and/or biofuel.

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BirA protein [Escherichia coli]
AF414937_1 Holocarboxylase Synthetase 1
    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    10      20      30      40      50      60
    -----MKDNT VPLKLIALLA NGEFHSGEQL GETLGMRAA INKHIQTLRD WGVDFVFTVPG
    MEAVRSTTTT L SNFHLNLT L RSLKPLRL SFSFSASAME SDASCSLVLC GKSSVETEVA

    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    70      80      90      100     110     120
    KGYSLPEPIQ LIN-----AKQILG QLD----- GGSVAVLPIV
    KGLKNKNSLK LPDNTKVS LI LESEAKNLVK DDDNSFNLSL FMNSIITHRF GRFLIWSPLR

    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    130     140     150     160     170     180
    DSTNQYLIDR IGEIKSGDAC VAEYQQAGRG RRGKWFSPF GANLYLSMFW RLEQGPAAAI
    SSTHDVSHN FSELPVGSVC VTDIQFKGRG RTKNVWESPK GCIMYS-FTL EMEDGRVPL

    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    190     200     210     220     230     240
    GLSLVIGIVM AEVLRLKGLAD K-----VRV KWPNDLYLQD RKLAILVEL TGKTGDAAI
    -IQYVVS LAV TEAVKDVCDK KGLPYIDVKI KWPNDLYVNG LKVGILCTS TYRS-KKFNV

    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    250     260     270     280     290     300
    VIGAGINMAM RRVEESVVNQ GWITLQEAGI NIDRNTLAAM LIRELRAALE LFEQEG LAP-
    SVGVGLNVDN G-QPTTCINA VLKGMAPESN LKREEILGA FFHKFEKFFD LFMDQGFKSL

    .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
    310     320     330     340     350     360
    ---YLSRWEK LDNFINRPVK LIIGDKEIFG ISRGIDKQGA LLLEQDG--- -IIKPPWMGGE
    EELYRTWLH SEQRVIVEDK VEDQVVQNVV TIQGLTSSGY LLAVGDDNQ M YELHPDGN SF

    .....|.....| .
    370
    ISLRSAEK--- -
    DFFKGLVRRK I

```

Fig. 1

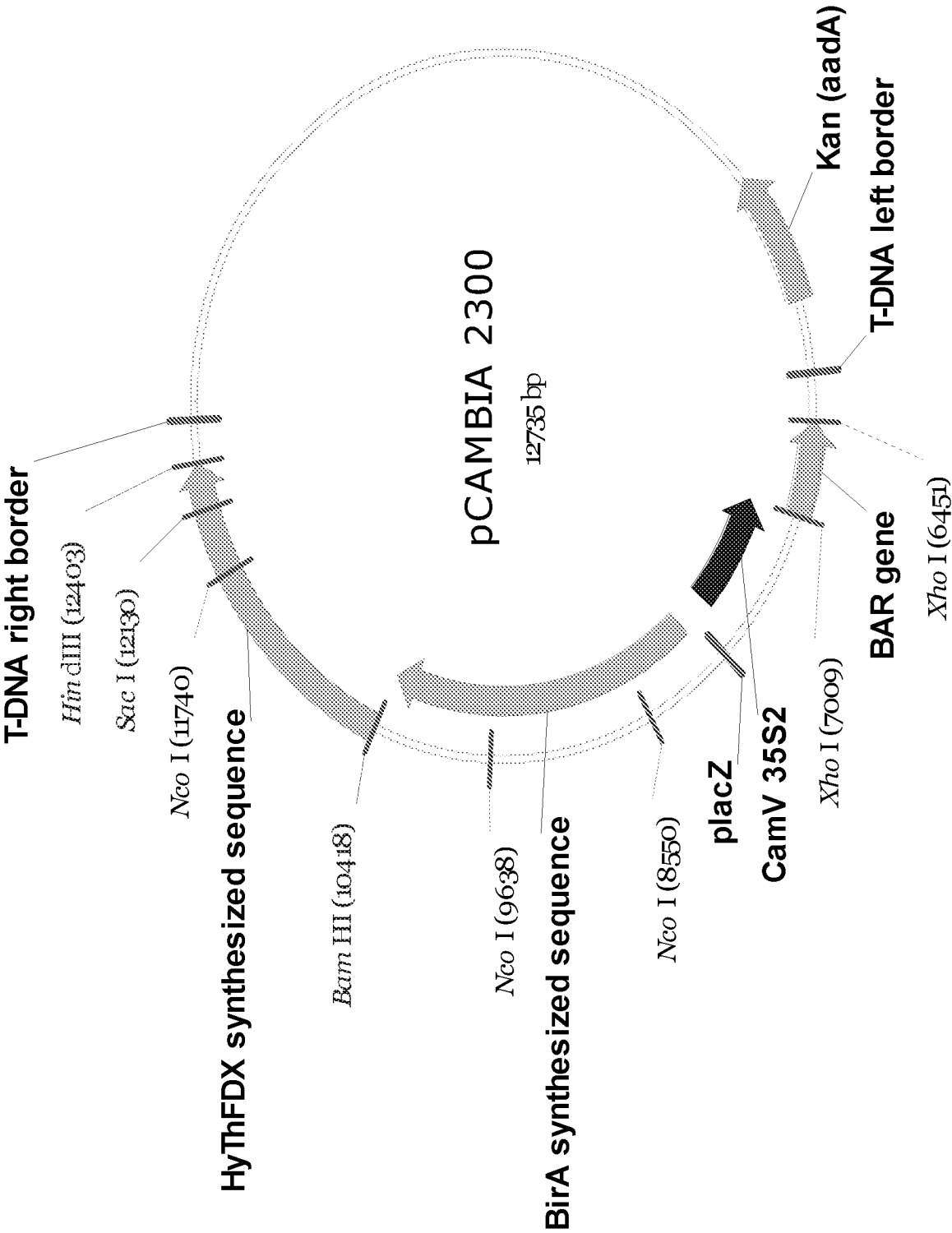


Fig. 2

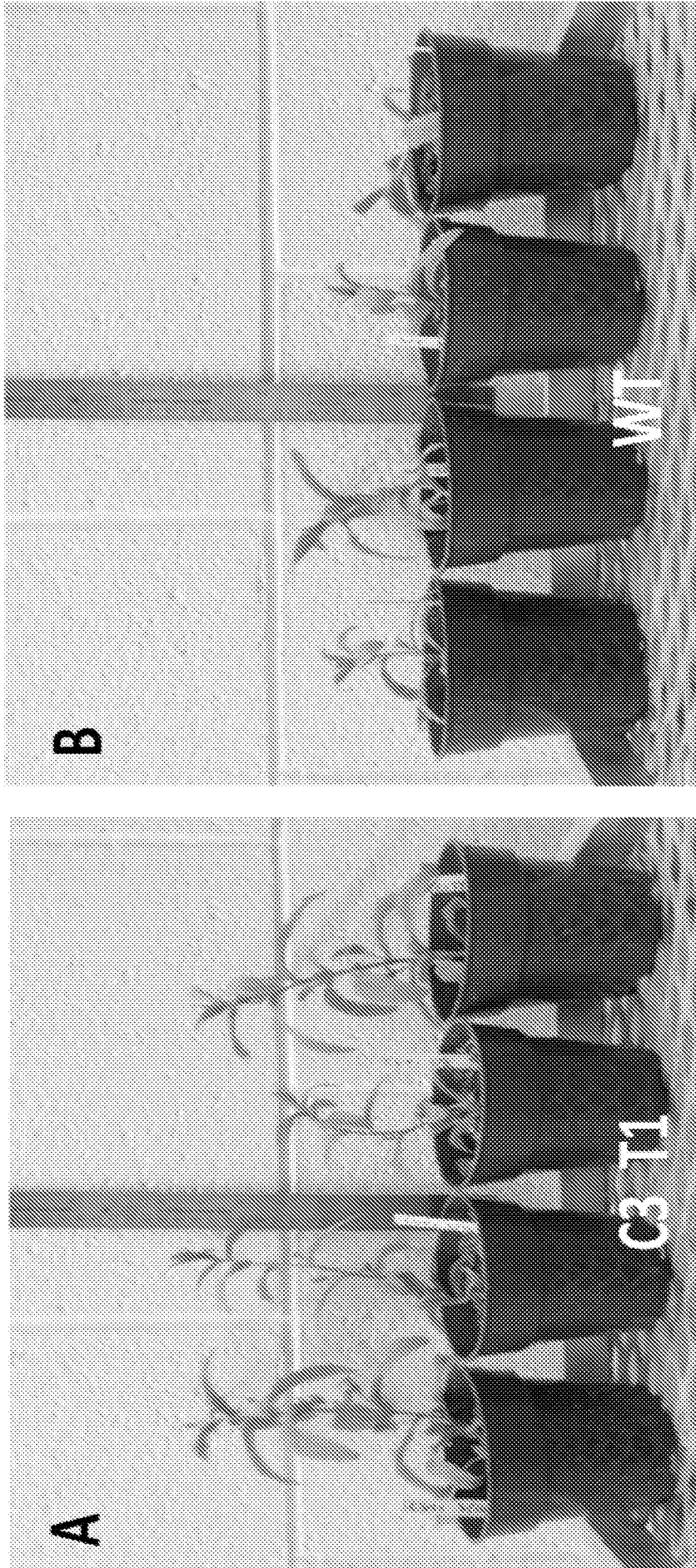


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/043064

A. CLASSIFICATION OF SUBJECT MATTER (see extra sheet) 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 15/52, 15/82, 5/04, A01H 5/00, 5/10, A23L 7/00 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) PatSearch, Espacenet, PATENTSCOPE, RUPTO, E-Library, Google Scholar, PubMed, USPTO, ScienceDirect		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CA 2892551 A1 (NORTH CAROLINA STATE UNIVERSITY) 05.06.2014, abstract, p.10, claims	1, 5, 9, 10, 12-17, 19-23, 25-27
A		2, 4, 6, 8, 11, 18, 24
Y	WO 2010/034652 A1 (BASF PLANT SCIENCE GMBH et al.) 01.04.2010, abstract, paragraph [0095], claims	1, 5, 9, 10, 12-17, 19-23, 25-27
Y	WANG D et al. Epigenome profiling of specific plant cell types using a streamlined INTACT protocol and ChIP-seq. Methods Mol Biol, Feb 2015, Vol. 1284, pp.3-25, especially abstract	9, 15, 19-21, 25
Y	CA 2770550 A1 (PLANT BIOSCIENCE LTD) 17.02.2011, abstract, claims	3, 7
Y	LIN YH et al. Expression of plant ferredoxin-like protein (PFLP) enhances tolerance to heat stress in Arabidopsis thaliana, New Biotechnology, 2015 Mar 25, Vol.32, no.2, pp.235-242, especially abstract	3, 7
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 07 December 2016 (07.12.2016)		Date of mailing of the international search report 08 December 2016 (08.12.2016)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37		Authorized officer E.Tereshkina Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/043064

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

According to Rule 13.2 of the Regulations under the PCT the claimed inventions does not meet the requirement of unity of invention *a priori*, therefore independent claims 1-8, 16-19, 22-26 form several groups of inventions, with are not linked as to form a single general inventive concept.

The special technical feature of independent claims 1, 3, 5, 7, 17, 23 (all completely), 16, 22, 26 (all partially) is a heterologous polynucleotide encoding a ferredoxin polypeptide.

The special technical feature of independent claims 2, 4, 6, 8, 18, 24 (all completely), 16, 22, 26 (all partially) is a heterologous polynucleotide encoding a biotin ligase polypeptide.

The special technical features of independent claims 19, 25 (all completely), 26 (partially) are a heterologous polynucleotide encoding a ferredoxin polypeptide and a heterologous polynucleotide encoding a biotin ligase polypeptide.

Hence, claims comprise 3 groups of inventions, namely:

- 1 invention – claims 1, 3, 5, 7, 17, 23 (all completely), 9-16, 20, 22, 26, 27 (all partially).
- 2 invention – claims 2, 4, 6, 8, 18, 24 (all completely), 11-16, 21, 22, 26, 27 (all partially).
- 3 invention – claims 19, 25 (all completely), 20, 21, 26, 27(all partially).

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
Classification of subject matter

International application No.

PCT/US 2016/043064

C12N 15/52 (2006.01)

C12N 15/82 (2006.01)

C12N 5/04 (2006.01)

A01H 5/00 (2006.01)

A01H 5/10 (2006.01)

A23L 7/00 (2006.01)