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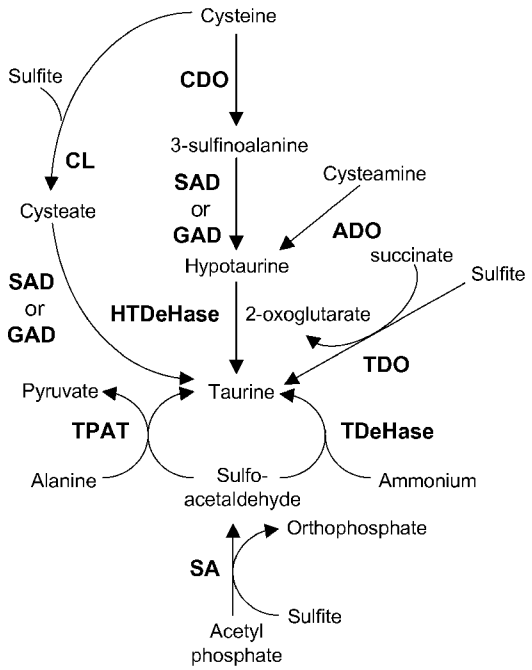
(54) Title: METHOD FOR THE BIOSYNTHESIS OF TAURINE OR HYPOTAURINE IN CELLS

FIGURE 1



(57) Abstract: The present invention describes an approach to increase taurine or hypotaurine production in prokaryotes or eukaryotes. More particularly, the invention relates to genetic transformation of organisms with genes that encode proteins that catalyze the conversion of cysteine to taurine, methionine to taurine, cysteamine to taurine, or alanine to taurine. The invention describes methods for the use of polynucleotides that encode functional cysteine dioxygenase (CDO), cysteine dioxygenase (CDO) and sulfinoalanine decarboxylase (SAD) or glutamate decarboxylase (GAD), cysteamine dioxygenase (ADO), taurine-pyruvate aminotransferase (TPAT), TPAT and sulfoacetaldehyde acetyltransferase (SA), taurine dioxygenase (TDO) or the small (ssTDeHase) and large subunits of taurine dehydrogenase (lsTDeHase) polypeptides in plants to increase taurine, hypotaurine or taurine precursor production. The preferred embodiment of the invention is in plants but other organisms may be used. Increased taurine production in plants will enhance plant growth and development, yield, or tolerance to biotic and/or abiotic stresses and could be used as nutraceutical, pharmaceutical, or therapeutic compounds or as a supplement in animal feed.

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METHODS FOR THE BIOSYNTHESIS OF TAURINE OR HYPOTAURINE IN CELLS

CROSS REFERENCE TO RELATED APPLICATION(S)

[01] This application claims the benefit of U.S. Patent Application No. 61/257,240 filed November 2, 2009 and U.S. Patent Application No.: 61/263,548 filed November 23, 2009, the disclosures of which are incorporated herein in their entirety by reference.

FIELD OF THE INVENTION

[02] The present invention is in the field of recombinant production of taurine.

BACKGROUND OF THE INVENTION

Taurine as a plant growth stimulator

[03] Exogenous application of taurine has been reported to increase crop harvest, yield, and biomass (1). Applications of taurine by foliar spray, soil and roots application, and seed immersion increase crop production and seedling growth (1). Exogenous applications of taurine have also been shown to increase photosynthetic capacity of isolated plant cells (protoplasts and chloroplasts) (1). Increased taurine production in plants can enhance plant growth and development, yield, or tolerance to biotic and/or abiotic stresses. Increased yield, growth, or biomass may be a result of increased nitrogen flow, sensing, uptake, storage, transport or nitrogen use efficiency. Increased yield, growth or biomass may also be a result of increased carbon metabolism due to increased photosynthesis or increased carbohydrate metabolism by increased sucrose production and/or transport or increase biosynthesis or mobilization of starch, or oil. Increased yield, growth or biomass may also be associated with increased phosphorus uptake, transport or utilization. Increased yield, growth or biomass may also be associated with increased sulfur or sulfate uptake, transport or utilization. Increased yield, growth or biomass may also be associated with increased water uptake, transport, utilization or water-use-efficiency.

Increased yield, growth or biomass may also be due to changes in the cell cycle modifications that improve growth rates and may increase early vigor and accelerate maturation leading to improved yield. Increased yield, growth or biomass may also be due to changes in the production of hormones or signaling molecules that regulate and improve plant growth and development leading to improvements in yield and biotic or abiotic stress tolerance. Increases in carbon, nitrogen, phosphorus, or sulfate flow, sensing, uptake, storage, transport or efficiency may improve seed quality for starch, oil or protein content. Increased yield, growth or biomass may also be a result of increased tolerance to abiotic stress such as changes in osmotic conditions, oxidative damage, drought, salt, cold, freezing, heat, UV light or light intensity. Increased yield, growth or biomass may also be a result of increased tolerance to biotic stress such as challenges, infection or insult from pests, pathogens, bacteria, microbes, viruses, viroids, microorganisms, invertebrates, insects, nematodes, or vertebrate. Increased yield, growth or biomass may be a result of increased tolerance to abiotic stresses such as changes in osmotic conditions or light intensity, oxidative damage, drought, salt, cold, freezing, heat, or UV radiation.

Taurine is an essential compound for animals

[04] Taurine is essential for human neonatal development (2) and plays an important role in brain development (3, 4). Taurine is involved in the modulation of intracellular calcium homeostasis (5, 6) and may balance glutamate activity, protecting neurons against glutamate excitotoxicity (7, 8). Taurine is also an osmoregulator (9). Taurine is essential for heart function (10), protects the integrity of hepatic tissue (11), and plays a role in photoprotection (12).

Taurine as a pharmaceutical or therapeutic

[05] Taurine is used as a pharmaceutical and therapeutic. Taurine has been used in the treatment of cardiovascular diseases (13, 14), elevated blood pressure (15), seizure disorders (16), hepatic disorders (17), and alcoholism (18) and may be useful in the

treament of diabetes (19), Alzheimer's disease (20), and ocular disorders (21). Taurine has been shown to prevent obesity (22) and control cholesterol (23, 24). Taurine acts as an antioxidant and protects against toxicity of various substances (25-27). Taurine has been shown to prevent oxidative stress induced by exercise (28), and is used in energy drinks to improve performance (29). Taurine can also be used in topical applications to treat dermatological conditions (30).

Taurine as a dietary supplement

[06] Taurine is biosynthesized in most animals and can be found in meat and seafood. Those who do not eat these foods regularly (e.g., vegetarians) or do not produce sufficient levels of taurine, e.g., cats (31), must acquire it through dietary supplement. Trout that are fed all-plant protein diets must acquire dietary taurine for normal growth (32).

Metabolic pathways that synthesize taurine

[07] With few exceptions (33, 34), taurine is found in plants only in low levels (35), and the metabolic pathway for taurine and hypotaurine has not yet been identified in plants. Several metabolic pathways that synthesize taurine and hypotaurine have been identified in animals and bacteria (Figure 1). In animals, cysteine and oxygen are converted into 3-sulfinoalanine by cysteine dioxygenase (CDO). 3-sulfinoalanine is converted into hypotaurine by sulfinoalanine decarboxylase (SAD) or glutamate decarboxylase (GAD). Hypotaurine is converted into taurine either by the activity of hypotaurine dehydrogenase (HTDeHase) or by a spontaneous conversion. Cysteamine (2-aminoethanethiol) and oxygen are converted into hypotaurine by cysteamine dioxygenase (ADO), and hypotaurine is converted into taurine. Alternatively cysteine and sulfite are converted into cysteate and hydrogen sulfide by cysteine lyase (cysteine sulfite lyase or cysteine hydrogen-sulfide-lyase). Cysteate is converted into taurine by SAD or GAD. In bacteria, the compound 2-sulfoacetaldehyde is synthesized from acetyl phosphate and sulfite by sulfoacetaldehyde acetyltransferase (SA). Alanine and 2-sulfoacetaldehyde are converted into taurine and pyruvate by taurine-pyruvate aminotransferase (TPAT). In addition,

sulfoacetaldehyde and ammonia (or ammonium) are converted into taurine and water in the presence of ferrocytochrome C by taurine dehydrogenase. Sulfite, aminoacetaldehyde, carbon dioxide and succinate are converted into taurine, 2-oxoglutarate and oxygen by taurine dioxygenase (TDO).

SUMMARY OF THE INVENTION

[08] The invention provides methods and compositions for taurine or taurine precursor production in organisms. More particularly, the invention encompasses the use of polynucleotides that encode in plants functional (1) cysteine dioxygenase (CDO), (2) CDO and sulfinoalanine decarboxylase (SAD) or glutamate decarboxylase (GAD), (3) cysteamine dioxygenase (ADO), (4) taurine-pyruvate aminotransferase (TPAT), (5) TPAT and sulfoacetaldehyde acetyltransferase (SA), (6) taurine dehydrogenase (TDeHase) or (7) taurine dioxygenase (TDO). The invention provides methods for transforming plants and constructing vector constructs and other nucleic acid molecules for use therein. The transgenic plants will have increased levels of taurine or taurine-precursors for enhanced plant growth and development, yield, or tolerance to biotic and/or abiotic stresses and can be used to provide nutraceuticals or pharmaceuticals for improving physical or mental performance, antioxidative activity, or therapeutic compounds in the treatment of conditions including congestive heart failure, high blood pressure, hepatitis, high cholesterol, diabetes, fibrosis, epilepsy, autism, attention deficit-hyperactivity disorder, retinal disorders, alcoholism, or as a food supplement in animal feed.

[09] The invention provides isolated cells comprising exogenous DNA which expresses enzymes of taurine biosynthetic pathways. In one embodiment, an isolated cell comprises two separate expression cassettes. A first expression cassette comprises a first promoter operably linked to a first polynucleotide, and a second expression cassette comprises a second promoter operably linked to a second polynucleotide. In some embodiments, the first polynucleotide encodes cysteine dioxygenase (CDO) and the second polynucleotide encodes sulfinoalanine decarboxylase (SAD). In other embodiments the first

polynucleotide encodes cysteine dioxygenase (CDO) and the second polynucleotide encodes glutamate decarboxylase (GAD). In still other embodiments, the first polynucleotide encodes taurine-pyruvate aminotransferase (TPAT) and the second polynucleotide encodes sulfoacetaldehyde acetyltransferase (SA). In yet other embodiments the first polynucleotide encodes a small subunit of taurine dehydrogenase (ssTDeHase) and the second polynucleotide encodes a large subunit of taurine dehydrogenase (lsTDeHase).

[10] Some isolated cells of the invention comprise exogenous DNA which comprises a single expression cassette. The single expression cassette comprises a promoter operably linked to a polynucleotide which encodes (i) CDO and SAD; (ii) CDO and GAD; (iii) TPAT; (iv) TPAT and SA; or (v) ssTDeHase and lsTDeHase.

[11] Other isolated cells of the invention are plant cells which comprise exogenous DNA which comprises a promoter operably linked to a polynucleotide. The polynucleotide encodes CDO, ADO, or taurine dioxygenase (TDO).

[12] The invention also provides plant storage organs comprising isolated cells of the invention; transgenic seeds with a genome comprising exogenous DNA encoding one or more of CDO, SAD, GAD, ADO, TPAT, SA, TDO, or TDeHase, and transgenic plants grown from the transgenic seeds.

[13] The invention provides methods of altering a property of a transgenic plant of the invention by contacting the transgenic plant with an agent which increases sulfur or nitrogen concentration in cells of the transgenic plant.

[14] The invention also provides pharmaceutical compositions and nutritional supplements comprising an extract of a transgenic plant of the invention, and feeds comprising a component, which can be one or more of the plant storage organs, transgenic seeds, and transgenic plants of the invention.

[15] In one embodiment of the invention polynucleotides encoding functional CDO and SAD or GAD enzymes are used to transform plant cells or to transform plants. Inventive methods produce plants that have advantages of enhanced taurine production, that result in plants with enhanced plant growth characteristics, survival characteristics and/or tolerance to environmental or other plant stresses and increase nutritional, pharmaceutical, or therapeutic value. Plants are genetically modified in accordance with the invention to introduce into the plant a polynucleotide that encodes a CDO enzyme and/or a polynucleotide that encodes a SAD or GAD that functions in the formation of hypotaurine or taurine in the plant.

[16] Another embodiment of the invention describes the use of ADO, TPAT, TDeHase, or TDO to produce hypotaurine or taurine in plants.

[17] Another embodiment of the invention describes the use of TPAT and SA to produce taurine in plants.

[18] Another embodiment of the invention describes the use of polynucleotides that encode polypeptides for functional CDO, CDO and SAD or GAD, ADO, TPAT, SA, TDeHase or TDO expressed in eukaryotes or prokaryotes or in eukaryotic or prokaryotic cells, for hypotaurine or taurine production.

BRIEF DESCRIPTION OF THE FIGURE

[19] Figure 1 shows taurine biosynthetic pathways.

DETAILED DESCRIPTION OF THE INVENTION

[20] The present invention provides methods and materials for the production of taurine (2-aminoethanesulfonic acid) in cells and living organisms. In preferred embodiments, the invention provides methods for the genetic transformation of organisms, preferably plants, with genes that encode proteins that catalyze the conversion of cysteine to taurine, methionine to taurine, cysteamine to taurine, or alanine to taurine. The invention also

provides methods of using plants with increased levels of endogenous taurine or taurine derivatives such as hypotaurine to improve plant growth, development and performance, that is to increase plant size, biomass, yield or tolerance to biotic or abiotic stress. The invention also provides methods of using plants with elevated levels of endogenous taurine or taurine derivatives such as hypotaurine as a food- or feed-supplement, dietary supplement, or as a component of a health supplement or therapy.

[21] The present invention describes the methods for the synthesis of DNA constructs for taurine or taurine precursor production from polynucleotides and vectors and the methods for making transformed organisms including plants, photosynthetic organisms, microbes, invertebrates, and vertebrates. The present invention is unique in that it describes a method to produce plants that have advantages of enhanced taurine production and that result in plants with enhanced plant growth characteristics, survival characteristics and/or tolerance to environmental or other plant stresses and increased nutritional, pharmaceutical, or therapeutic value.

[22] The present invention describes the insertion of the taurine biosynthetic pathway in organisms where the pathway does not exist or has not clearly been identified. The invention describes methods for the use of polynucleotides that encode functional cysteine dioxygenase (CDO) and sulfinoalanine decarboxylase (SAD) or glutamate decarboxylase (GAD), cysteamine dioxygenase (ADO), taurine-pyruvate aminotransferase (TPAT), TPAT and sulfoacetaldehyde acetyltransferase (SA), taurine dehydrogenase (TDeHase) or taurine dioxygenase (TDO) in plants. The preferred embodiment of the invention is in plants but other organisms may be used.

Enzymes of taurine biosynthetic pathways

[23] Examples of amino acid sequences of enzymes of taurine biosynthetic pathways are provided in the sequence listing: SEQ ID NO:3 and SEQ ID NO:4 (CDO); SEQ ID NO:7 and SEQ ID NO:8 (SAD); SEQ ID NO:11 and SEQ ID NO:12 (GAD); SEQ ID NO:18 (TPAT); SEQ ID NO:20 (SA); SEQ ID NO:22 (ssTDeHase); SEQ ID NO:22

(lsTDeHase); SEQ ID NO:13 and SEQ ID NO:14 (ADO); and SEQ ID NO:26 (TDO). The invention is not limited to the use of these amino acid sequences. Those of ordinary skill in the art know that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and effectively provide similar function. For example, the amino acid sequences for CDO, SAD, GAD, or ADO from zebra fish (*Danio rerio)* or TPAT, SA, ssTDeHase or lsTDeHase from *Roseobacter denitrificans* or TDO from *Escherichia coli* may differ to a certain degree from the amino acid sequences of CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO in another species and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity, and it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have activity.

[24] Another manner in which similarity may exist between two amino acid sequences is where there is conserved substitution between a given amino acid of one group, such as a non-polar amino acid, an uncharged polar amino acid, a charged polar acidic amino acid, or a charged polar basic amino acid, with an amino acid from the same amino acid group. For example, it is known that the uncharged polar amino acid serine may commonly be substituted with the uncharged polar amino acid threonine in a polypeptide without substantially altering the functionality of the polypeptide. Whether a given substitution will affect the functionality of the enzyme may be determined without undue experimentation using synthetic techniques and screening assays known to one with ordinary skill in the art.

[25] One of ordinary skill in the art will recognize that changes in the amino acid sequences, such as individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is "sufficiently similar" when the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7 or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO activity is generally at least 40%, 50%, 60%, 70%, 80% or 90%, preferably 60-90% of the native protein for the native substrate. Tables of conserved substitution provide lists of functionally similar amino acids.

[26] The following three groups each contain amino acids that are conserved substitutions for one another: (1) Alanine (A), Serine (S), Threonine (T); (2) Aspartic acid (D), Glutamic acid (E); and (3) Asparagine (N), Glutamine (Q);

Suitable polynucleotides for CDO, SAD, GAD, ADO, TPAT, SA, TDO, ssTDeHase, and lsTDeHase

[27] As examples, suitable polynucleotides encoding enzymes of taurine biosynthetic pathways are described below. The invention is not limited to use of these sequences, however. In fact, any nucleotide sequence which encodes an enzyme of a taurine biosynthetic pathway can be used in an expression vector to produce that enzyme recombinantly.

[28] Suitable polynucleotides for CDO are provided in SEQ ID NO:1 and SEQ ID NO:2 Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:1 or SEQ ID NO:2 by hybridization under low stringency conditions, moderate

stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:1 or SEQ ID NO:2 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:3 or SEQ ID NO:4 when it used as a reference for sequence comparison.

[29] Suitable polynucleotides for SAD are provided in SEQ ID NO:5 and SEQ ID NO:6. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:5 or SEQ ID NO:6 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:5 or SEQ ID NO:6 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:7 or SEQ ID NO:8 when it is used as a reference for sequence comparison.

[30] Suitable polynucleotides for GAD are provided in SEQ ID NO:9 and SEQ ID NO:10. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:9 or SEQ ID NO:10 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:9 or SEQ ID NO:10 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:11 or SEQ ID NO:12 when it used as a reference for sequence comparison.

[31] Suitable polynucleotides for ADO are provided in SEQ ID NO:13 and SEQ ID NO:14. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:13 or SEQ ID NO:14 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:13 or SEQ ID NO:14 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:15 or SEQ ID NO:16 when it used as a reference for sequence comparison.

[32] A suitable polynucleotide for TPAT is provided in SEQ ID NO:17. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:17 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:17 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:18 when it used as a reference for sequence comparison.

[33] A suitable polynucleotide for SA is provided in SEQ ID NO:19. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:19 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:19 when it used as a

reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:20 when it used as a reference for sequence comparison.

[34] A suitable polynucleotide for ssTDeHase is provided in SEQ ID NO:21. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:21 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:21 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:22 when it used as a reference for sequence comparison.

[35] A suitable polynucleotide for lsTDeHase is provided in SEQ ID NO:23. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:23 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:23 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:24 when it used as a reference for sequence comparison.

[36] A suitable polynucleotide for TDO is provided in SEQ ID NO:25. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:25 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in

accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:25 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:26 when it used as a reference for sequence comparison.

[37] Another embodiment of the invention is a polynucleotide (*e.g.*, a DNA construct) that encodes a protein that functions as a CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO and selectively hybridizes to either SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25 respectively. Selectively hybridizing sequences typically have at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 100% sequence identity with each other.

[38] Another embodiment of the invention is a polynucleotide that encodes a polypeptide that has substantial identity to the amino acid sequence of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 50-100%, preferably at least 55%, preferably at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

[39] The process of encoding a specific amino acid sequence may involve DNA sequences having one or more base changes (*i.e.*, insertions, deletions, substitutions) that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not eliminate the functional properties of the polypeptide encoded by the DNA sequence.

[40] It is therefore understood that the invention encompasses more than the specific polynucleotides encoding the proteins described herein. For example, modifications to a

sequence, such as deletions, insertions, or substitutions in the sequence, which produce "silent" changes that do not substantially affect the functional properties of the resulting polypeptide are expressly contemplated by the present invention. Furthermore, because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill in the art will recognize that each amino acid has more than one codon, except for methionine and tryptophan that ordinarily have the codons AUG and UGG, respectively. It is known by those of ordinary skill in the art, "universal" code is not completely universal. Some mitochondrial and bacterial genomes diverge from the universal code, *e.g.*, some termination codons in the universal code specify amino acids in the mitochondria or bacterial codes. Thus each silent variation of a nucleic acid, which encodes a polypeptide of the present invention, is implicit in each described polypeptide sequence and incorporated in the descriptions of the invention.

[41] It is understood that alterations in a nucleotide sequence, which reflect the degeneracy of the genetic code, or which result in the production of a chemically equivalent amino acid at a given site, are contemplated. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product.

[42] Nucleotide changes which result in alteration of the amino-terminal and carboxy-terminal portions of the encoded polypeptide molecule would also not generally be expected to alter the activity of the polypeptide. In some cases, it may in fact be desirable to make mutations in the sequence in order to study the effect of alteration on the biological activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art.

[43] When the nucleic acid is prepared or altered synthetically, one of ordinary skill in the art can take into account the known codon preferences for the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC-content preferences of monocotyledonous plants or dicotyledonous plants, as these preferences have been shown to differ (36). An alternative approach to the generation of variants of the sequences is to use random recombination techniques such as "DNA shuffling" (37). An alternative method to modify the sequences is by rapid molecular evolution methods such as a staggered extension process (38).

Cloning techniques

[44] For purposes of promoting an understanding of the principles of the invention, reference will now be made to particular embodiments of the invention and specific language will be used to describe the same. The materials, methods and examples are illustrative only and not limiting. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. Specific terms, while employed below and defined at the end of this section, are used in a descriptive sense only and not for purposes of limitation. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art (39-46).

[45] A suitable polynucleotide for use in accordance with the invention may be obtained by cloning techniques using cDNA or genomic libraries, DNA, or cDNA from bacteria which are available commercially or which may be constructed using standard methods known to persons of ordinary skill in the art. Suitable nucleotide sequences may be isolated from DNA libraries obtained from a wide variety of species by means of nucleic acid hybridization or amplification methods, such as polymerase chain reaction (PCR) procedures, using as probes or primers nucleotide sequences selected in accordance with the invention.

[46] Furthermore, nucleic acid sequences may be constructed or amplified using chemical synthesis. The product of amplification is termed an amplicon. Moreover, if the particular nucleic acid sequence is of a length that makes chemical synthesis of the entire length impractical, the sequence may be broken up into smaller segments that may be synthesized and ligated together to form the entire desired sequence by methods known in the art. Alternatively, individual components or DNA fragments may be amplified by PCR and adjacent fragments can be amplified together using fusion-PCR (47), overlap-PCR (48) or chemical (de novo) synthesis (49-53) by methods known in the art.

[47] A suitable polynucleotide for use in accordance with the invention may be constructed by recombinant DNA technology, for example, by cutting or splicing nucleic acids using restriction enzymes and mixing with a cleaved (cut with a restriction enzyme) vector with the cleaved insert (DNA of the invention) and ligated using DNA ligase. Alternatively amplification techniques, such as PCR, can be used, where restriction sites are incorporated in the primers that otherwise match the nucleotide sequences (especially at the 3′ ends) selected in accordance with the invention. The desired amplified recombinant molecule is cut or spliced using restriction enzymes and mixed with a cleaved vector and ligated using DNA ligase. In another method, after amplification of the desired recombinant molecule, DNA linker sequences are ligated to the 5′ and 3′ ends of the desired nucleotide insert with ligase, the DNA insert is cleaved with a restriction enzyme that specifically recognizes sequences present in the linker sequences and the desired

vector. The cleaved vector is mixed with the cleaved insert, and the two fragments are ligated using DNA ligase. In yet another method, the desired recombinant molecule is amplified with primers that have recombination sites (*e.g.* Gateway) incorporated in the primers, that otherwise match the nucleotide sequences selected in accordance with the invention. The desired amplified recombinant molecule is mixed with a vector containing the recombination site and recombinase, the two molecules are ligated together by recombination.

[48] The recombinant expression cassette or DNA construct includes a promoter that directs transcription in a plant cell, operably linked to the polynucleotide encoding a CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase or lsTDeHase. In various aspects of the invention described herein, a variety of different types of promoters are described and used. As used herein, a polynucleotide is "operably linked" to a promoter or other nucleotide sequence when it is placed into a functional relationship with the promoter or other nucleotide sequence. The functional relationship between a promoter and a desired polynucleotide insert typically involves the polynucleotide and the promoter sequences being contiguous such that transcription of the polynucleotide sequence will be facilitated. Two nucleic acid sequences are further said to be operably linked if the nature of the linkage between the two sequences does not (1) result in the introduction of a frame-shift mutation; (2) interfere with the ability of the promoter region sequence to direct the transcription of the desired nucleotide sequence, or (3) interfere with the ability of the desired nucleotide sequence to be transcribed by the promoter sequence region. Typically, the promoter element is generally upstream (i.e., at the 5′ end) of the nucleic acid insert coding sequence.

[49] While a promoter sequence can be ligated to a coding sequence prior to insertion into a vector, in other embodiments, a vector is selected that includes a promoter operable in the host cell into which the vector is to be inserted. In addition, certain preferred vectors have a region that codes a ribosome binding site positioned between the promoter and the site at which the DNA sequence is inserted so as to be operatively associated with the DNA

sequence of the invention to produce the desired polypeptide, *i.e.*, the DNA sequence of the invention in-frame.

Suitable promoters

[50] A wide variety of promoters are known to those of ordinary skill in the art as are other regulatory elements that can be used alone or in combination with promoters. A wide variety of promoters that direct transcription in plants cells can be used in connection with the present invention. For purposes of describing the present invention, promoters are divided into two types, namely, constitutive promoters and non-constitutive promoters. Constitutive promoters are classified as providing for a range of constitutive expression. Thus, some are weak constitutive promoters, and others are strong constitutive promoters. Non-constitutive promoters include tissue-preferred promoters, tissue-specific promoters, cell-type specific promoters, and inducible-promoters.

[51] Of particular interest in certain embodiments of the present invention are inducible-promoters that respond to various forms of environmental stresses, or other stimuli, including, for example, mechanical shock, heat, cold, salt, flooding, drought, salt, anoxia, pathogens, such as bacteria, fungi, and viruses, and nutritional deprivation, including deprivation during times of flowering and/or fruiting, and other forms of plant stress. For example, the promoter selected in alternate forms of the invention, can be a promoter is induced by one or more, but not limiting to one of the following, abiotic stresses such as wounding, cold, dessication, ultraviolet-B (54), heat shock (55) or other heat stress, drought stress or water stress. The promoter may further be one induced by biotic stresses including pathogen stress, such as stress induced by a virus (56) or fungi (57, 58), stresses induced as part of the plant defense pathway (59) or by other environmental signals, such as light (60), carbon dioxide (61, 62), hormones or other signaling molecules such as auxin, hydrogen peroxide and salicylic acid (63, 64), sugars and gibberellin (65) or abscissic acid and ethylene (66).

[52] In other embodiments of the invention, tissue-specific promoters are used. Tissue-specific expression patterns as controlled by tissue- or stage-specific promoters that include, but is not limited to, fiber-specific, green tissue-specific, root-specific, stem-specific, and flower-specific. Examples of the utilization of tissue-specific expression includes, but is not limited to, the expression in leaves of the desired peptide for the protection of plants against foliar pathogens, the expression in roots of the desired peptide for the protection of plants against root pathogens, and the expression in roots or seedlings of the desired peptide for the protection of seedlings against soil-borne pathogens. In many cases, however, protection against more than one type of pathogen may be sought, and expression in multiple tissues will be desirable.

[53] Although many promoters from dicotyledons have been shown to be operational in monocotyledons and vice versa, ideally dicotyledonous promoters are selected for expression in dicotyledons, and monocotyledonous promoters are selected for expression in monocotyledons. There are also promoters that control expression of genes in green tissue or for genes involved in photosynthesis from both monocotyledons and dicotyledons such as the maize from the phosphenol carboxylase gene (67). There are suitable promoters for root specific expression (68, 69). A promoter selected can be an endogenous promoter, *i.e.* a promoter native to the species and or cell type being transformed. Alternatively, the promoter can be a foreign promoter, which promotes transcription of a length of DNA of viral, microbes, bacterial or eukaryotic origin, invertebrates, vertebrates including those from plants and plant viruses. For example, in certain preferred embodiments, the promoter may be of viral origin, including a cauliflower mosaic virus promoter (CaMV), such as CaMV 35S or19S, a figwort mosaic virus promoter (FMV 35S), or the coat protein promoter of tobacco mosaic virus (TMV). The promoter may further be, for example, a promoter for the small subunit of ribulose-1, 3-biphosphate carboxylase. Promoters of bacterial origin (microbe promoters) include the octopine synthase promoter, the nopaline synthase promoter and other promoters derived from native Ti plasmids (70).

[54] The promoters may further be selected such that they require activation by other elements known to those of ordinary skill in the art, so that production of the protein encoded by the nucleic acid sequence insert may be regulated as desired. In one embodiment of the invention, a DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention is used to make a transformed plant that selectively increases the level of the desired polypeptide of the invention in response to a signal. The term "signal" is used to refer to a condition, stress or stimulus that results in or causes a non-constitutive promoter to direct expression of a coding sequence operably linked to it. To make such a plant in accordance with the invention, a DNA construct is provided that includes a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention. The construct is incorporated into a plant genome to provide a transformed plant that expresses the polynucleotide in response to a signal.

[55] In alternate embodiments of the invention, the selected promoter is a tissue-preferred promoter, a tissue-specific promoter, a cell-type-specific promoter, an inducible promoter or other type of non-constitutive promoter. It is readily apparent that such a DNA construct causes a plant transformed thereby to selectively express the gene for the desired polypeptide of the invention. Therefore under specific conditions or in certain tissue- or cell-types the desired polypeptide will be expressed. The result of this expression in the plant depends upon the activity of the promoter and in some cases the conditions of the cell or cells in which it is expressed.

[56] It is understood that the non-constitutive promoter does not continuously produce the transcript or RNA of the invention. But in this embodiment the selected promoter for inclusion of the invention advantageously induces or increases transcription of gene for the desired polypeptide of the invention in response to a signal, such as an environmental cue or other stress signal including biotic and/or abiotic stresses or other conditions.

[57] In another embodiment of the invention, a DNA construct comprising a plant GAD promoter operably linked to polynucleotides that encode the desired polypeptide of the

invention is used to make a transformed plant that selectively increases the transcript or RNA of the desired polypeptide of the invention in the same cells, tissues, and under the environmental conditions that express a plant glutamate decarboxylase. It is understood to those of ordinary skill in the art that the regulatory sequences that comprise a plant promoter driven by RNA polymerase II reside in the region approximately 2900 to 1200 basepairs up-stream (5′) of the translation initiation site or start codon (ATG). For example, the full-length promoter for the nodule-enhanced PEP carboxylase from alfalfa is 1277 basepairs prior to the start codon (71), the full-length promoter for cytokinin oxidase from orchid is 2189 basepairs prior to the start codon (72), the full-length promoter for ACC oxidase from peach is 2919 basepairs prior to the start codon (73), full-length promoter for cytokinin oxidase from orchid is 2189 basepairs prior to the start codon, full-length promoter for glutathione peroxidase1 from *Citrus sinensis* is 1600 basepairs prior to the start codon (74), and the full-length promoter for glucuronosyltransferase from cotton is 1647 basepairs prior to the start codon (75). Most full-length promoters are 1700 basepairs prior to the start codon. The accepted convention is to describe this region (promoter) as –1700 to –1, where the numbers designate the number of basepairs prior to the "A" in the start codon. In this embodiment of the invention that the region of –2000 to –1 basepairs 5′ to a plant GAD is operably linked to a polynucleotide for the said encoded peptide to make a transformed plant that selectively expresses the polynucleotide or increases the level of the said protein where the plant GAD is expressed or accumulates. A plant GAD promoter is the -2000 to –1 basepair region genes that include, but is not limited to, the five *Arabidopsis thaliana* GADs (AtGAD) (76), petunia GAD (77), tomato GAD (78), tobacco GAD (79), rice (80), barely, poplar, soybean, mustard, orange, *Medicago truncatula*, grape and pine. Those of ordinary skill in the art can either digest the desired region using restriction enzymes and ligase to clone the plant GAD promoters or use amplification, such as PCR, techniques with the incorporation of restriction or recombination sites to clone the plant GAD promoters 5′ to the desired polynucleotide. A plant GAD promoter for these purposes normally means the following regions upstream (5′) to the start codon between

–200 to –1 basepairs, preferably at least between –500 to –1 basepairs, preferably at least between –1000 to –1 basepairs, more preferably at least between –1500 to –1 basepairs, and most preferably at –2000 to –1 basepairs.

[58] In another embodiment of the invention, a DNA construct comprising a plant glutamate receptor promoter operably linked to polynucleotides that encode the desired polypeptide of the invention is used to make a transformed plant that selectively increases the transcript or RNA of the desired polypeptide of the invention in the same cells, tissues, and under the environmental conditions that express a plant glutamate receptor. It is understood to those of ordinary skill in the art that the regulatory sequences that comprise a plant promoter driven by RNA polymerase II reside in the region approximately 2900 to 1200 basepairs up-stream (5′) of the translation initiation site or start codon (ATG). A plant glutamate receptor promoter is the -2000 to –1 basepair region genes that include, but is not limited to, the 20 *Arabidopsis thaliana* glutamate receptors (AtGLRs or AtGluRs) and 23 rice glutamate receptors. The promoters for the following AtGLRs genes, 1.1, 2.1, 3.1 (81), 3.2 (note this is designated as GLR2 in the manuscript; (82), and 3.4 (83) have been shown to control specific cell-type, tissue-type, developmental and environmental expression patterns in plants. Those of ordinary skill in the art can either digest the desired region using restriction enzymes and ligase to clone the plant glutamate promoters or use amplification, such as PCR, techniques with the incorporation of restriction or recombination sites to clone the plant glutamate receptor promoters 5′ to the desired polynucleotide. A plant glutamate receptor promoter for these purposes normally means the following regions upstream (5′) to the start codon between –200 to –1 basepairs, preferably at least between –500 to –1 basepairs, preferably at least between –1000 to –1 basepairs, more preferably at least between –1500 to –1 basepairs, and most preferably at –2000 to –1 basepairs.

[59] In another embodiment of the invention, a DNA construct comprising a plant sulphate transporter promoter operably linked to polynucleotides that encode the desired polypeptide of the invention is used to make a transformed plant that selectively increases

the transcript or RNA of the desired polypeptide of the invention in the same cells, tissues, and under the environmental conditions that express a plant sulphate transporter. It is understood to those of ordinary skill in the art that the regulatory sequences that comprise a plant promoter driven by RNA polymerase II reside in the region approximately 2900 to 1200 basepairs up-stream (5′) of the translation initiation site or start codon (ATG). A plant sulphate transporter promoter is the -2000 to -1 basepair region genes that include, but is not limited to, the *Arabidopsis thaliana* sulphate transporters (SULTR or AtSULTR). The promoters for the following SULTR genes, SULTR1;1, SULTR1;2 (84), SULTR 1;3; (85), SULTR2;1 (86), and SULTR3;5 (87) have been shown to control specific cell-type, tissue-type, developmental and environmental expression patterns in plants. Those of ordinary skill in the art can either digest the desired region using restriction enzymes and ligase to clone the plant glutamate promoters or use amplification, such as PCR, techniques with the incorporation of restriction or recombination sites to clone the plant sulphate transporter promoters 5′ to the desired polynucleotide. A plant sulphate transporter promoter for these purposes normally means the following regions upstream (5′) to the start codon between -200 to -1 basepairs, preferably at least between -500 to -1 basepairs, preferably at least between -1000 to -1 basepairs, more preferably at least between -1500 to -1 basepairs, and most preferably at -2000 to -1 basepairs.

Suitable vectors

[60] A wide variety of vectors may be employed to transform a plant, plant cell or other cells with a construct made or selected in accordance with the invention, including high- or low-copy number plasmids, phage vectors and cosmids. Such vectors, as well as other vectors, are well known in the art. Representative T-DNA vector systems (70, 88) and numerous expression cassettes and vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are known and available (89). The vectors can be chosen such that operably linked promoter and polynucleotides that encode the desired polypeptide of the invention are incorporated into the genome of the

plant. Although the preferred embodiment of the invention is expression in plants or plant cells, other embodiments may include expression in prokaryotic or eukaryotic photosynthetic organisms, microbes, invertebrates or vertebrates.

[61] It is known by those of ordinary skill in the art that there exist numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. There are many commercially available recombinant vectors to transform a host plant or plant cell. Standard molecular and cloning techniques (43, 46, 90) are available to make a recombinant expression cassette that expresses the polynucleotide that encodes the desired polypeptide of the invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made. In brief, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter, followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high-level expression of a cloned gene, it is desirable to construct expression vectors that contain, at the minimum, a strong promoter, such as ubiquitin, to direct transcription, a ribosome-binding site for translational initiation, and a transcription/translation terminator.

[62] One of ordinary skill to the art recognizes that modifications could be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, targeting or to direct the location of the polypeptide in the host, or for the purification or detection of the polypeptide by the addition of a "tag" as a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, additional amino acids (tags) placed on either terminus to create a tag, additional nucleic acids to insert a restriction site or a termination.

[63] In addition to the selection of a suitable promoter, the DNA constructs requires an appropriate transcriptional terminator to be attached downstream of the desired gene of the invention for proper expression in plants. Several such terminators are available and known to persons of ordinary skill in the art. These include, but are not limited to, the tml from CaMV and E9 from rbcS. Another example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. A wide variety of available terminators known to function in plants can be used in the context of this invention. Vectors may also have other control sequence features that increase their suitability. These include an origin of replication, enhancer sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, selectable markers and RNA stability signal. Origin of replication is a gene sequence that controls replication of the vector in the host cell. Enhancer sequences cooperate with the promoter to increase expression of the polynucleotide insert coding sequence. Enhancers can stimulate promoter activity in host cell. An example of specific polyadenylation sequence in higher eukaryotes is ATTTA. Examples of plant polyadenylation signal sequences are AATAAA or AATAAT. RNA splice sites are sequences that ensure accurate splicing of the transcript. Selectable markers usually confer resistance to an antibiotic, herbicide or chemical or provide color change, which aid the identification of transformed organisms. The vectors also include a RNA stability signal, which are 3′-regulatory sequence elements that increase the stability of the transcribed RNA (91, 92).

[64] In addition, polynucleotides that encode a CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO can be placed in the appropriate plant expression vector used to transform plant cells. The polypeptide can then be isolated from plant callus or the transformed cells can be used to regenerate transgenic plants. Such transgenic plants can be harvested, and the appropriate tissues can be subjected to large-scale protein extraction and purification techniques.

[65] The vectors may include another polynucleotide insert that encodes a peptide or polypeptide used as a “tag” to aid in purification or detection of the desired protein. The

additional polynucleotide is positioned in the vector such that upon cloning and expression of the desired polynucleotide a fusion, or chimeric, protein is obtained. The tag may be incorporated at the amino or carboxy terminus. If the vector does not contain a tag, persons with ordinary skill in the art know that the extra nucleotides necessary to encode a tag can be added with the ligation of linkers, adaptors, or spacers or by PCR using designed primers. After expression of the peptide the tag can be used for purification using affinity chromatography, and if desired, the tag can be cleaved with an appropriate enzyme. The tag can also be maintained, not cleaved, and used to detect the accumulation of the desired polypeptide in the protein extracts from the host using western blot analysis. In another embodiment, a vector includes the polynucleotide for the tag that is fused in-frame to the polynucleotide that encodes a functional CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO to form a fusion protein. The tags that may be used include, but are not limited to, Arg-tag, calmodulin-binding peptide, cellulose-binding domain, DsbA, c-myc-tag, glutathione S-transferase, FLAG-tag, HAT-tag, His-tag, maltose-binding protein, NusA, S-tag, SBP-tag, Strep-tag, and thioredoxin (Trx-Tag). These are available from a variety of manufacturers Clontech Laboratories, Takara Bio Company GE Healthcare, Invitrogen, Novagen Promega and QIAGEN.

[66] The vector may include another polynucleotide that encodes a signal polypeptide or signal sequence ("subcellular location sequence") to direct the desired polypeptide in the host cell, so that the polypeptide accumulates in a specific cellular compartment, subcellular compartment, or membrane. The specific cellular compartments include the apoplast, vacuole, plastids chloroplast, mitochondrion, peroxisomes, secretory pathway, lysosome, endoplasmic reticulum, nucleus or Golgi apparatus. A signal polypeptide or signal sequence is usually at the amino terminus and normally absent from the mature protein due to protease that removes the signal peptide when the polypeptide reaches its final destination. Signal sequences can be a primary sequence located at the N-terminus (93-96), C-terminus (97, 98) or internal (99-101) or tertiary structure (101). If a signal polypeptide or signal sequence to direct the polypeptide does not exist on the vector, it is

expected that those of ordinary skill in the art can incorporate the extra nucleotides necessary to encode a signal polypeptide or signal sequence by the ligation of the appropriate nucleotides or by PCR. Those of ordinary skill in the art can identify the nucleotide sequence of a signal polypeptide or signal sequence using computational tools. There are numerous computational tools available for the identification of targeting sequences or signal sequence. These include, but are not limited to, TargetP (102, 103), iPSORT (104), SignalP (105), PrediSi (106), ELSpred (107) HSLpred (108) and PSLpred (109), MultiLoc (110), SherLoc (111), ChloroP (112), MITOPROT (113), Predotar (114) and 3D-PSSM (115). Additional methods and protocols are discussed in the literature (110).

Fusion of two gene products

[67] Two gene products can be fused together to increase the efficiency of an enzymatic reaction conducted by two enzymes (116-118). The two genes can be fused in-frame to be expressed as a single gene product with or without a linker. The linker can be a sequence that encodes a "tag" or a peptide.

Transformation of host cells

[68] Transformation of a plant can be accomplished in a wide variety of ways within the scope of a person of ordinary skill in the art. In one embodiment, a DNA construct is incorporated into a plant by (i) transforming a cell, tissue or organ from a host plant with the DNA construct; (ii) selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; (iii) regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and (iv) selecting a regenerated whole plant that expresses the polynucleotide. Many methods of transforming a plant, plant tissue or plant cell for the construction of a transformed cell are suitable. Once transformed, these cells can be used to regenerate transgenic plants (119).

[69] Those of ordinary skill in the art can use different plant gene transfer techniques found in references for, but not limited to, the electroporation (120-124), microinjection (125, 126), lipofection (127), liposome or spheroplast fusions (128-130), Agrobacterium (131), direct gene transfer (132), T-DNA mediated transformation of monocots (133), T-DNA mediated transformation of dicots); (134, 135), microprojectile bombardment or ballistic particle acceleration (136-139), chemical transfection including CaCl2 precipitation, polyvinyl alcohol, or poly-L-ornithine (140), silicon carbide whisker methods (141, 142), laser methods (143, 144), sonication methods (145-147), polyethylene glycol methods (148), and vacuum infiltration (149) and transbacter (150).

[70] In one embodiment of the invention, a transformed host cell may be cultured to produce a transformed plant. In this regard, a transformed plant can be made, for example, by transforming a cell, tissue or organ from a host plant with an inventive DNA construct; selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and selecting a regenerated whole plant that expresses the polynucleotide.

[71] A wide variety of host cells may be used in the invention, including prokaryotic and eukaryotic host cells. These cells or organisms may include microbes, invertebrate, vertebrates or photosynthetic organisms. Preferred host cells are eukaryotic, preferably plant cells, such as those derived from monocotyledons, such as duckweed, corn, rice, sugarcane, wheat, bent grass, rye grass, Bermuda grass, Blue grass, and Fescue, or dicotyledons, including canola, cotton, camelina, lettuce, rapeseed, radishes, cabbage, sugarbeet, peppers, broccoli, potatoes and tomatoes, and legumes such as soybeans and bush beans.

[72] One embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the CDO construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional SAD gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector; and

6. transform the vector containing the SAD construct into a plant or plant cell carrying a CDO construct or one that expresses a functional CDO gene product.

[73] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for the functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the CDO construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional SAD gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector;

6. transform the vector containing the SAD construct into a plant or plant cell; and

7. Sexually cross a plant (or fuse cells) carrying a CDO construct or one that expresses a functional CDO with a plant (or cells) carrying a SAD construct or one that expresses a functional SAD gene product.

[74] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. In the same vector, operably link a promoter to the 5′ end of the polynucleotide for the functional CDO gene product;

2. operably link a promoter to the 5′ end of the polynucleotide for the functional SAD gene product;

3. insert the two polynucleotides into the vector in such a manner that both polynucleotides are expressed by one promoter or each polynucleotide is expressed by one promoter; and

4. transform the vector containing the CDO and SAD constructs into a plant or plant cell.

[75] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the CDO construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional GAD gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector; and

6. transform the vector containing the GAD construct into a plant or plant cell carrying a CDO construct or one that expresses a functional CDO gene product.

[76] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for the functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the CDO construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional GAD gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector;

6. transform the vector containing the GAD construct into a plant or plant cell; and

7. Sexually cross a plant (or fuse cells) carrying a CDO construct or one that expresses a functional CDO with a plant (or cells) carrying a GAD construct or one that expresses a functional GAD gene product.

[77] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. In the same vector, operably link a promoter to the 5′ end of the polynucleotide for the functional CDO gene product;

2. operably link a promoter to the 5′ end of the polynucleotide for the functional GAD gene product;

3. insert the two polynucleotides into the vector in such a manner that both polynucleotides are expressed by one promoter or each polynucleotide is expressed by one promoter; and

4. transform the vector containing the CDO and GAD constructs into a plant or plant cell.

[78] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the CDO construct into a plant or plant cell.

[79] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional SAD gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the SAD construct into a plant or plant cell.

[80] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional ADO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the ADO construct into a plant or plant cell.

[81] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional TPAT gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the TPAT construct into a plant or plant cell.

[82] One embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional TPAT gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the TPAT construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional SA gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector; and

6. transform the vector containing the TPAT construct into a plant or plant cell carrying a SA construct or one that expresses a functional SA gene product.

[83] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for the functional TPAT gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the TPAT construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional SA gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector;

6. transform the vector containing the SA construct into a plant or plant cell; and

7. Sexually cross a plant (or fuse cells) carrying a TPAT construct or one that expresses a functional TPAT with a plant (or cells) carrying a SA construct or one that expresses a functional SA gene product.

[84] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. In the same vector, operably link a promoter to the 5′ end of the polynucleotide for the functional TPAT gene product;

2. operably link a promoter to the 5′ end of the polynucleotide for the functional SA gene product;

3. insert the two polynucleotides into the vector in such a manner that both polynucleotides are expressed by one promoter or each polynucleotide is expressed by one promoter; and

4. transform the vector containing the TPAT and SA construct into a plant or plant cell.

[85] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional small subunit of TDeHase (ssTDeHase) gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the ssTDeHase construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional large subunit of TDeHase (lsTDeHase) gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector; and

6. transform the vector containing the lsTDeHase construct into a plant or plant cell carrying a ssTDeHase construct or one that expresses a functional ssTDeHase gene product.

[86] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for the functional ssTDeHase gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector;

3. transform the vector containing the ssTDeHase construct into a plant or plant cell;

4. operably link a promoter to the 5′ end of the polynucleotide for the functional lsTDeHase gene product;

5. insert the polynucleotide construct (from step 4 above) into a vector;

6. transform the vector containing the lsTDeHase construct into a plant or plant cell; and

7. Sexually cross a plant (or fuse cells) carrying a ssTDeHase construct or one that expresses a functional ssTDeHase with a plant (or cells) carrying a lsTDeHase construct or one that expresses a functional lsTDeHase gene product.

[87] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. In the same vector, operably link a promoter to the 5′ end of the polynucleotide for the functional ssTDeHase gene product;

2. operably link a promoter to the 5′ end of the polynucleotide for the functional lsTDeHase gene product;

3. insert the two polynucleotides into the vector in such a manner that both polynucleotides are expressed by one promoter or each polynucleotide is expressed by one promoter; and

4. transform the vector containing the ssTDeHase and lsTDeHase construct into a plant or plant cell.

[88] Another embodiment of the invention is a method for the production of taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional TDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the TDO construct into a plant or plant cell.

[89] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO fused in-frame to a functional SAD gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the CDO-SAD construct into a plant or plant cell.

[90] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO fused with a linker in-frame to a functional SAD gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the CDO-linker-SAD construct into a plant or plant cell.

[91] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional SAD fused in-frame to a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the SAD-CDO construct into a plant or plant cell.

[92] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional SAD fused with a linker in-frame to a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the SAD-linker-CDO construct into a plant or plant cell.

[93] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO fused in-frame to a functional GAD gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the CDO-GAD construct into a plant or plant cell.

[94] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional CDO fused with a linker in-frame to a functional GAD gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the CDO-linker-GAD construct into a plant or plant cell.

[95] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional GAD fused in-frame to a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the GAD-CDO construct into a plant or plant cell.

[96] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional GAD fused with a linker in-frame to a functional CDO gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the GAD-linker-CDO construct into a plant or plant cell.

[97] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional TPAT fused in-frame to a functional SA gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the TPAT-SA construct into a plant or plant cell.

[98] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional TPAT fused with a linker in-frame to a functional SA gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the TPAT-linker-SA construct into a plant or plant cell.

[99] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional SA fused in-frame to a functional TPAT gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the SA-TPAT construct into a plant or plant cell.

[100] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional SA fused with a linker in-frame to a functional TPAT gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the SA-linker-TPAT construct into a plant or plant cell.

[101] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional ssTDeHase fused in-frame to a functional lsTDeHase gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the ssTDeHase-lsTDeHase construct into a plant or plant cell.

[102] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional ssTDeHase fused with a linker in-frame to a functional lsTDeHase gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the ssTDeHase-linker-lsTDeHase construct into a plant or plant cell.

[103] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional lsTDeHase fused in-frame to a functional ssTDeHase gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the lsTDeHase-ssTDeHase construct into a plant or plant cell.

[104] Another embodiment of the invention is a method for the production of hypotaurine or taurine by the following steps:

1. operably link a promoter to the 5′ end of the polynucleotide for a functional lsTDeHase fused with a linker in-frame to a functional ssTDeHase gene product;

2. insert the polynucleotide construct (from step 1 above) into a vector; transform the vector containing the lsTDeHase-linker-ssTDeHase construct into a plant or plant cell.

Suitable plants

[105] The methods described above may be applied to transform a wide variety of plants, including decorative or recreational plants or crops, but are particularly useful for treating commercial and ornamental crops. Examples of plants that may be transformed in the present invention include, but are not limited to, Acacia, alfalfa, algae, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beech, beet, Bermuda grass, bent grass, blackberry, blueberry, Blue grass, broccoli, Brussels sprouts, cabbage, camelina, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, duckweed, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, fescue, figs, forest trees, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, maize, mango, melon, mushroom, nectarine, nut, oat, okra, onion, orange, an ornamental

plant, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, rye grass, seaweed, scallion, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, switchgrass, tangerine, tea, tobacco, tomato, turf, turnip, a vine, watermelon, wheat, yams, and zucchini. Other suitable hosts include bacteria, fungi, algae and other photosynthetic organisms, and animals including vertebrate and invertebrates.

[106] Once transformed, the plant may be treated with other "active agents" either prior to or during the exposure of the plant to stress to further decrease the effects of plant stress. "Active agent," as used herein, refers to an agent that has a beneficial effect on the plant or increases production of amino acid production by the plant. For example, the agent may have a beneficial effect on the plant with respect to nutrition, and the resistance against, or reduction of, the effects of plant stress. Some of these agents may be precursors of end products for reaction catalyzed by CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase or lsTDeHase. These compounds could promote growth, development, biomass and yield, and change in metabolism. In addition to the twenty amino acids that are involved in protein synthesis specifically sulfur containing amino acids methionine, and cysteine, other amino acids such as glutamate, glutamine, serine, alanine and glycine, sulfur containing compounds such as fertilizer, sulfite, sulfide, sulfate, taurine, hypotaurine, cysteate, 2-sulfacetaldehyde, homotaurine, homocysteine, cystathionine, N-acetyl thiazolidine 4 carboxylic acid (ATCA), glutathione, or bile, or other non-protein amino acids, such as GABA, citrulline and ornithine, or other nitrogen containing compounds such as polyamines may also be used to activate CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO. Depending on the type of gene construct or recombinant expression cassette, other metabolites and nutrients may be used to activate CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase, or TDO. These include, but are not limited to, sugars, carbohydrates, lipids, oligopeptides, mono- (glucose, arabinose, fructose, xylose, and ribose) di- (sucrose and trehalose) and polysaccharides,

carboxylic acids (succinate, malate and fumarate) and nutrients such as phosphate, molybdate, or iron.

[107] Accordingly, the active agent may include a wide variety of fertilizers, pesticides and herbicides known to those of ordinary skill in the art (151). Other greening agents fall within the definition of "active agent" as well, including minerals such as calcium, magnesium and iron. The pesticides protect the plant from pests or disease and may be either chemical or biological and include fungicides, bactericides, insecticides and anti-viral agents as known to those of ordinary skill in the art.

[108] In some embodiments properties of a transgenic plant are altered using an agent which increases sulfur concentration in cells of the transgenic plant, such as fertilizer, sulfur, sulfite, sulfide, sulfate, taurine, hypotaurine, homotaurine, cysteate, 2-sulfacetaldehyde, N-acetyl thiazolidine 4 carboxylic acid (ATCA), glutathione, and bile. In other embodiments, the agent increases nitrogen concentration. Amino acids, either naturally occurring in proteins (*e.g.*, cysteine, methionine, glutamate, glutamine, serine, alanine, or glycine) or which do not naturally occur in proteins (*e.g.*, GABA, citrulline, or ornithine) and/or polyamines can be used for this purpose.

Expression in Prokaryotes

[109] The use of prokaryotes as hosts includes strains of *E. coli.* However, other microbial strains including, but not limited to, *Bacillus* (152) and *Salmonella* may also be used. Commonly used prokaryotic control sequences include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences. Commonly used prokaryotic promoters include the beta lactamase (153), lactose (153), and tryptophan (154) promoters. The vectors usually contain selectable markers to identify transfected or transformed cells. Some commonly used selectable markers include the genes for resistance to ampicillin, tetracycline, or chloramphenicol. The vectors are typically a plasmid or phage. Bacterial cells are transfected or transformed with the plasmid vector DNA. Phage DNA can be infected with phage vector particles or

transfected with naked phage DNA. The plasmid and phage DNA for the vectors are commercially available from numerous vendors known to those of ordinary skill in the art.

Expression in non-plant eukaryotes

[110] The present invention can be expressed in a variety of eukaryotic expression systems such as yeast, insect cell lines, and mammalian cells which are known to those of ordinary skill in the art. For each host system there are suitable vectors that are commercially available (*e.g.*, Invitrogen, Startagene, GE Healthcare Life Sciences). The vectors usually have expression control sequences, such as promoters, an origin of replication, enhancer sequences, termination sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and selectable markers. Synthesis of heterologous proteins in yeast is well known to those of ordinary skill in the art (155, 156). The most widely used yeasts are *Saccharomyces cerevisiae* and *Pichia pastoris*. Insect cell lines that include, but are not limited to, mosquito larvae, silkworm, armyworm, moth, and *Drosophila* cell lines can be used to express proteins of the present invention using baculovirus-derived vectors. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell lines.

[111] A protein of the present invention, once expressed in any of the non-plant eukaryotic systems can be isolated from the organism by lysing the cells and applying standard protein isolation techniques to the lysates or the pellets. The monitoring of the purification process can be accomplished by using western blot techniques or radioimmunoassay of other standard immunoassay techniques.

Pharmaceutical compositions

[112] The invention provides pharmaceutical compositions which comprise extracts of one or more transgenic plants described above. Plant extracts containing taurine and hypotaurine can be used to synthesize or manufacture homotaurine or other taurine derivatives (157, 158), taurine-conjugates (159) or taurine-polymers (160) that may have a wide range of commercial and medicinal applications (161). Some taurine derivatives can function as organogelators (162) or dyes (163) and can be used in nanosensor synthesis (164). Some taurine derivatives have anticonvulsant (157) or anti-cancer (165) properties. Other taurine derivatives are used in the treatment of alcoholism (166, 167). Taurine-conjugated carboxyethylester-polyrotaxanes increase anticoagulant activity (168). Taurine-containing polymers may increase wound healing (169, 170). Taurine linked polymers such as poly gamma-glutamic acid-sulfonates are biodegradable and may have applications in the development of drug delivery systems, environmental materials, tissue engineering, and medical materials (171). Extracts from taurine-containing plants may be used in pharmaceutical or medicinal compositions to deliver taurine, hypotaurine, taurine-conjugates, or taurine-polymers for use in the treatment of congestive heart failure, high blood pressure, hepatitis, high cholesterol, fibrosis, epilepsy, autism, attention deficit-hyperactivity disorder, retinal degeneration, diabetes, and alcoholism. It is also used to improve mental performance and as an antioxidant.

[113] Pharmaceutically acceptable vehicles of taurine, taurine derivatives, taurine-conjugates, or taurine-polymers are tablets, capsules, gel, ointment, film, patch, powder or dissolved in liquid form.

Nutritional Supplements and Feeds

[114] Transgenic plants containing taurine or hypotaurine may be consumed or used to make extracts for nutritional supplements. Transgenic plant parts that have elevated levels of taurine or hypotaurine may be used for human consumption. The plant parts may include but are not limited to leaves, stalks, stems, tubers, stolons, roots, petioles, cotyledons,

seeds, fruits, grain, strover, nuts, flowers, petioles, pollen, buds, or pods. Extracts from transgenic plants containing taurine or hypotaurine may be used as nutritional supplements, as an antioxidant or to improve physical or mental performance. The extracts may be used in the form of a liquid, powder, capsule or tablet.

[115] Transgenic plants containing taurine or hypotaurine may be used as fish or animal feed or used to make extracts for the supplementation of animal feed. Plant parts that have elevated levels of taurine or hypotaurine may be used as animal or fish feed include but are not limited to leaves, stalks, stems, tubers, stolons, roots, petioles, cotyledons, seeds, fruits, grain, strover, nuts, flowers, petioles, buds, pods, or husks. Extracts from transgenic plants containing taurine or hypotaurine may be used as feed supplements in the form of a liquid, powder, capsule or tablet.

DEFINITIONS

[116] The term "polynucleotide" refers to a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, including deoxyribonucleic acid, ribonucleic acid, and derivatives thereof. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role. Unless otherwise indicated, nucleic acids or polynucleotide are written left to right in 5′ to 3′ orientation, Nucleotides are referred to by their commonly accepted single-letter codes. Numeric ranges are inclusive of the numbers defining the range.

[117] The terms "amplified" and "amplification" refer to the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification can be achieved by chemical synthesis using any of the following methods, such as solid-phase phosphoramidate technology or the polymerase chain reaction (PCR). Other amplification systems include the ligase chain reaction system, nucleic acid sequence based amplification, Q-*Beta* Replicase systems, transcription-based amplification system,

and strand displacement amplification. The product of amplification is termed an amplicon.

[118] As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase, either I, II or III, and other proteins to initiate transcription. Promoters include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as far as several thousand base pairs from the start site of transcription.

[119] The term "plant promoter" refers to a promoter capable of initiating transcription in plant cells.

[120] The term "microbe promoter" refers to a promoter capable of initiating transcription in microbes.

[121] The term "foreign promoter" refers to a promoter, other than the native, or natural, promoter, which promotes transcription of a length of DNA of viral, bacterial or eukaryotic origin, including those from microbes, plants, plant viruses, invertebrates or vertebrates.

[122] The term "microbe" refers to any microorganism (including both eukaryotic and prokaryotic microorganisms), such as fungi, yeast, bacteria, actinomycetes, algae and protozoa, as well as other unicellular structures.

[123] The term "plant" includes whole plants, and plant organs, and progeny of same. Plant organs comprise, *e.g.*, shoot vegetative organs/structures (*e.g.* leaves, stems and tubers), roots, flowers and floral organs/structures (*e.g.* bracts, sepals, petals, stamens, carpels, anthers and ovules), seed (including embryo, endosperm, and seed coat) and fruit (the mature ovary), plant tissue (*e.g.* vascular tissue, ground tissue, and the like) and cells (*e.g.* guard cells, egg cells, trichomes and the like). The class of plants that can be used in the

method of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, and multicellular algae. It includes plants of a variety of ploidy levels, including aneuploid, polyploid, diploid, haploid and hemizygous.

[124] The term "plant storage organ" includes roots, seeds, tubers, fruits, and specialized stems.

[125] The term "constitutive" refers to a promoter that is active under most environmental and developmental conditions, such as, for example, but not limited to, the CaMV 35S promoter and the nopaline synthase terminator.

[126] The term "tissue-preferred promoter" refers to a promoter that is under developmental control or a promoter that preferentially initiates transcription in certain tissues.

[127] The term "tissue-specific promoter" refers to a promoter that initiates transcription only in certain tissues.

[128] The term "cell-type specific promoter" refers to a promoter that primarily initiates transcription only in certain cell types in one or more organs.

[129] The term "inducible promoter" refers to a promoter that is under environmental control.

[130] The terms "encoding" and "coding"" refer to the process by which a polynucleotide, through the mechanisms of transcription and translation, provides the information to a cell from which a series of amino acids can be assembled into a specific amino acid sequence to produce a functional polypeptide, such as, for example, an active enzyme or ligand binding protein.

[131] The terms "polypeptide," "peptide," "protein" and "gene product" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally

occurring amino acid polymers. Amino acids may be referred to by their commonly known three-letter or one-letter symbols. Amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range.

[132] The terms "residue," "amino acid residue," and "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide. The amino acid may be a naturally occurring amino acid and may encompass known analogs of natural amino acids that can function in a similar manner as the naturally occurring amino acids.

[133] The terms "cysteine dioxygenase" and "CDO" refer to the protein (EC:1.13.11.20) that catalyzes the following reaction:

cysteine + oxygen = 3-sulfinoalanine

[134] NOTE: 3-sulfinoalanine is another name for cysteine sulfinic acid, cysteine sulfinate, 3-sulphino-L-alanine, 3-sulfino-alanine, 3-sulfino-L-alanine, L-cysteine sulfinic acid, L-cysteine sulfinic acid, cysteine hydrogen sulfite ester or alanine 3-sulfinic acid

[135] The terms "sulfinoalanine decarboxylase" and "SAD" refer to the protein (4.1.1.29) that catalyzes the following reaction:

3-sulfinoalanine = hypotaurine = CO_2

NOTE: SAD is another name for cysteine-sulfinate decarboxylase, L-cysteine sulfinic acid decarboxylase, cysteine-sulfinate decarboxylase, CADCase/CSADCase, CSAD, cysteic decarboxylase, cysteine sulfinic acid decarboxylase, cysteine sulfinate decarboxylase, sulfoalanine decarboxylase, sulphinoalanine decarboxylase, and 3-sulfino-L-alanine carboxy-lyase.

NOTE: the SAD reaction is also catalyzed by GAD (4.1.1.15) (glutamic acid decarboxylase or glutamate decarboxylase).

[136] Other names for hypotaurine are 2-aminoethane sulfinate, 2-aminoethylsulfinic acid, and 2-aminoethanesulfinic acid

[137] Other names for taurine are 2-aminoethane sulfonic acid, aminoethanesulfonate, L-taurine, taurine ethyl ester, and taurine ketoisocaproic acid 2-aminoethane sulfinate.

[138] The terms "cysteamine dioxygenase" and "ADO" refer to the protein (EC 1.13.11.19) that catalyzes the following reaction:

$$\text{2-aminoethanethiol} + O_2 = \text{hypotaurine}$$

[139] ADO is another name for 2-aminoethanethiol:oxygen oxidoreductase, persulfurase, cysteamine oxygenase, and cysteamine:oxygen oxidoreductase.

[140] Other names for 2-aminoethanethiol are cysteamine or 2-aminoethane-1-thiol, b-mercaptoethylamine, 22-mercaptoethylamine, decarboxycysteine, and thioethanolamine.

[141] The terms "taurine-pyruvate aminotransferase" and "TPAT" refer to the protein (EC 2.6.1.77) that catalyzes the following reaction:

$$\text{L-alanine} + \text{2-sulfoacetaldehyde} = \text{taurine} + \text{pyruvate}$$

[142] TPAT is another name for taurine transaminase or Tpa

[143] The terms "sulfoacetaldehyde acetyltransferase" and "SA" refer to the protein (EC:2.3.3.15) that catalyzes the following reaction:

$$\text{acetyl phosphate} + \text{sulfite} = \text{sulfoacetaldehyde} + \text{orthophosphate}$$

[144] SA is another name for acetyl-phosphate:sulfite S-acetyltransferase or Xsc

[145] The terms "taurine dehydrogenase" and "TDeHase" refer to the protein (EC:1.4.2.-) that catalyzes the following reaction:

$$\text{ammonia} + \text{2-sulfoacetaldehyde} = \text{taurine} + \text{water}$$

[146] TDeHase is another name for taurine:oxidoreductase, taurine:ferricytochrome-c oxidoreductase, tauX or tauY

[147] The terms "taurine dioxygenase" and "TDO" refer to the protein (EC:1.14.11.17) that catalyzes the following reaction:

sulfite + aminoacetaldehyde + succinate + CO_2 = taurine + 2-oxoglutarate + O_2

[148] TDO is another name for 2-aminoethanesulfonate dioxygenase, alpha-ketoglutarate-dependent taurine dioxygenase, taurine, 2-oxoglutarate:O2 oxidoreductase or tauD

[149] 2-oxoglutarate is another name for alpha-ketoglutarate

[150] The term "functional" with reference to CDO, SAD, GAD, ADO, TPAT, SA, ssTDeHase, lsTDeHase or TDO refers to peptides, proteins or enzymes that catalyze the CDO, SAD, GAD, ADO, TPAT, SA, TDeHase or TDO reactions, respectively.

[151] The term "recombinant" includes reference to a cell or vector that has been modified by the introduction of a heterologous nucleic acid. Recombinant cells express genes that are not normally found in that cell or express native genes that are otherwise abnormally expressed, underexpressed, or not expressed at all as a result of deliberate human intervention, or expression of the native gene may have reduced or eliminated as a result of deliberate human intervention.

[152] The term "recombinant expression cassette" refers to a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter.

[153] The term "transgenic plant" includes reference to a plant, which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is also used to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenic plants altered or created by sexual crosses or asexual propagation from the initial transgenic plant. The term "transgenic" does not encompass the alteration of the genome by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

[154] The term "vector" includes reference to a nucleic acid used in transfection or transformation of a host cell and into which can be inserted a polynucleotide.

[155] The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (*e.g.*, at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 100% sequence identity (*i.e.*, complementary) with each other.

[156] The terms "stringent conditions" and "stringent hybridization conditions" include reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (*e.g.*, at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which can be up to 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted

to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Optimally, the probe is approximately 500 nucleotides in length, but can vary greatly in length from less than 500 nucleotides to equal to the entire length of the target sequence.

[157] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt solution. Low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulfate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. High stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated (172), where the T_m = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions

can utilize a hybridization and/or wash at 1, 2, 3 or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill in the art will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. An extensive guide to the hybridization of nucleic acids is found in the scientific literature (90, 173). Unless otherwise stated, in the present application high stringency is defined as hybridization in 4X SSC, 5X Denhardt solution (5 g Ficoll, 5 g polyvinypyrrolidone, 5 g bovine serum albumin in 500ml of water), 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65°C, and a wash in 0.1X SSC, 0.1% SDS at 65°C.

[158] The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides or polypeptides: "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity."

[159] The term "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

[160] The term "comparison window" includes reference to a contiguous and specified segment of a polynucleotide sequence, where the polynucleotide sequence may be compared to a reference sequence and the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) when it is compared to the reference sequence for optimal alignment. The comparison window is usually at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100 or longer. Those of ordinary skill in the art understand that the inclusion of gaps in a polynucleotide

sequence alignment introduces a gap penalty, and it is subtracted from the number of matches.

[161] Methods of alignment of nucleotide and amino acid sequences for comparison are well known to those of ordinary skill in the art. The local homology algorithm, BESTFIT, (174) can perform an optimal alignment of sequences for comparison using a homology alignment algorithm called GAP (175), search for similarity using Tfasta and Fasta (176), by computerized implementations of these algorithms widely available on-line or from various vendors (Intelligenetics, Genetics Computer Group). CLUSTAL allows for the alignment of multiple sequences (177-179) and program PileUp can be used for optimal global alignment of multiple sequences (180). The BLAST family of programs can be used for nucleotide or protein database similarity searches. BLASTN searches a nucleotide database using a nucleotide query. BLASTP searches a protein database using a protein query. BLASTX searches a protein database using a translated nucleotide query that is derived from a six-frame translation of the nucleotide query sequence (both strands). TBLASTN searches a translated nucleotide database using a protein query that is derived by reverse-translation. TBLASTX search a translated nucleotide database using a translated nucleotide query.

[162] GAP (175) maximizes the number of matches and minimizes the number of gaps in an alignment of two complete sequences. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It also calculates a gap penalty and a gap extension penalty in units of matched bases. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package are 8 and 2, respectively. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 100. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match.

Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package is BLOSUM62 (181).

[163] Unless otherwise stated, sequence identity or similarity values refer to the value obtained using the BLAST 2.0 suite of programs using default parameters (182). As those of ordinary skill in the art understand that BLAST searches assume that proteins can be modeled as random sequences and that proteins comprise regions of nonrandom sequences, short repeats, or enriched for one or more amino acid residues, called low-complexity regions. These low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. Those of ordinary skill in the art can use low-complexity filter programs to reduce number of low-complexity regions that are aligned in a search. These filter programs include, but are not limited to, the SEG (183, 184) and XNU (185).

[164] The terms "sequence identity" and "identity" are used in the context of two nucleic acid or polypeptide sequences and include reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When the percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (*e.g.*, charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conserved substitutions, the percent sequence identity may be adjusted upwards to correct for the conserved nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity." Scoring for a conservative substitution allows for a partial rather than a full mismatch (186), thereby increasing the percentage sequence similarity.

[165] The term "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise gaps (additions or deletions) when compared to the reference sequence for optimal alignment. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[166] The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has between 50-100% sequence identity, preferably at least 50% sequence identity, preferably at least 60% sequence identity, preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of ordinary skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 50-100%. Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each low stringency conditions, moderate stringency conditions or high stringency conditions. Yet another indication that two nucleic acid sequences are substantially identical is if the two polypeptides immunologically cross-react with the same antibody in a western blot, immunoblot or ELISA assay.

[167] The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with between 55-100% sequence identity to a reference sequence preferably at least 55% sequence identity, preferably 60% preferably 70%, more preferably 80%, most preferably at least 90% or 95% sequence identity to the reference

sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm (175). Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conserved substitution. Another indication that amino acid sequences are substantially identical is if two polypeptides immunologically cross-react with the same antibody in a western blot, immunoblot or ELISA assay. In addition, a peptide can be substantially identical to a second peptide when they differ by a non-conservative change if the epitope that the antibody recognizes is substantially identical.

REFERENCES

1. Suzuki *et al.,* 1989. United States Patent No. 4877447.
2. Sturman 1988. "Taurine in development." J Nutr, 118: 1169-1176.
3. Sturman *et al.,* 1980. "The biology of taurine in nutrition and development." Adv Nutr Res, 3: 231-299.
4. Chen *et al.,* 1998. "Effect of taurine on human fetal neuron cells: Proliferation and differentiation." Adv Exp Med Biol, 442: 397-403.
5. El Idrissi *et al.,* 1999. "Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism." J Neurosci, 19: 9459-9468.
6. El Idrissi *et al.,* 2003. "Taurine regulates mitochondrial calcium homeostasis." Adv Exp Med Biol, 526: 527-536.
7. Trenkner 1990. "Possible role of glutamate with taurine in neuron-glia interaction during cerebellar development." Prog Clin Biol Res, 351: 133-140.
8. Wu *et al.,* 2005. "Mode of action of taurine as a neuroprotector." Brain Res, 1038: 123-131.
9. Schaffer *et al.,* 2000. "Role of osmoregulation in the actions of taurine." Amino Acids, 19: 527-546.
10. Chapman *et al.,* 1993. "Taurine and the heart." Cardiovasc Res, 27: 358-363.
11. Tabassuma *et al.,* 2006. "Attenuation of tamoxifen-induced hepatotoxicity by taurine in mice." Clin Chim Acta, 370: 129-136.
12. Rocket *et al.,* 2007. "The osmolyte taurine protects against ultraviolet B radiation-induced immunosuppression." J Immunol, 179: 3604-3612.
13. Milei *et al.,* 1992. "Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularization." Am Heart J, 123: 339-345.
14. Militante *et al.,* 2002. "Treatment of hypertension with oral taurine." Endocrinology, 147: 3276-3284.

15. Fujita *et al.,* 1987. "Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension." Circulation, 75: 525-532.
16. McCown *et al.,* 1987. "Amino acid influences on seizures elicited within the inferior colliculus." J Pharmacol Exp Ther, 243: 603-608.
17. Matsuyama *et al.,* 1983. "The effect of taurine administration on patients with acute hepatitis." Prog Clin Biol Res, 125: 461-468.
18. Ikeda 1977. "Effects of taurine on alcohol withdrawal." Lancet, 2: 509.
19. Franconi *et al.,* 2004. "Is taurine beneficial in reducing risk factors for diabetes mellitus?" Neurochem Res, 29: 143-150.
20. Paula-Lima *et al.,* 2005. "Activation of GABAA receptors by taurine and muscimol blocks the neurotoxicity of [beta]-amyloid in rat hippocampal and cortical neurons." Neuropharmacology, 49: 1140-1148.
21. Nakamori *et al.,* 1993. "Quantitative evaluation of the effectiveness of taurine in protecting the ocular surface against oxidant." Chem Pharm Bull, 41: 335-338.
22. Zhang *et al.,* 2004. "Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects." Amino Acids, 26: 267-271.
23. Yokogoshi *et al.,* 1999. "Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet." J Nutr, 129: 1705-1712.
24. Yamamoto *et al.,* 2000. "Dietary taurine decreases hepatic secretion of cholesterol ester in rats fed a high-cholesterol diet." Pharmacology, 60: 27-33.
25. Green *et al.,* 1991. "Antioxidant role and subcellular location of hypotaurine and taurine in human neutrophils." Biochim Biophys Acta, 1073: 91-97.
26. Gürer *et al.,* 2001. "Antioxidant effect of taurine against lead-induced oxidative stress." Arch Environ Contam Toxicol, 41: 397-402.
27. Das *et al.,* 2008. "Taurine provides antioxidant defense against NaF-induced cytotoxicity in murine hepatocytes." Pathophysiology, 15: 181-190.
28. Zhang *et al.,* 2004. " Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men." Amino Acids, 26: 203-207.
29. Williams 2005. "Dietary supplements and sports performance: Amino acids." Journal of the International Society of Sports Nutrition, 2: 63-67.
30. da Silva *et al.,* 2008. "Penetration profile of taurine in the human skin and its distribution in skin layers." Pharm Res, 25: 1846-1850.
31. Knopf *et al.,* 1978. "Taurine: An essential nutrient for the cat." J Nutr, 108: 773-778.
32. Gibson *et al.,* 2007. "Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*)." Aquaculture, 269: 514-524.
33. Schweigen 1967. "Low-molecular-weight compounds in Macrocystis pyrifera, a marine algae." Arch Biochem Biophys, 118: 383-387.
34. Huxtable 1992. "Physiological actions of taurine." Physiol Rev, 72: 101-163.
35. Kataoka *et al.,* 1986. "Occurrence of taurine in plants." Agric Biol Chem, 50: 1887-1888.
36. Murray *et al.,* 1989. "Codon usage in plant genes." Nucleic Acids Research, 17: 477-498.
37. Stemmer 1997. United States Patent No. 5605793.
38. Short 1999. United States Patent No. 5965408.

39. Langenheim *et al.,* 1982. Botany: Plant Biology and its Relation to Human Affairs. New York: John Wiley & Sons Inc.
40. Vasil 1984. Cell Culture and Somatic Cell Genetics of Plants: Laboraory Procedures and Their Applications. Orlando: Academic Press.
41. Stanier *et al.,* 1986. The Microbial World. New Jersey: Prentice-Hall.
42. Dhringra *et al.,* 1985. Basic plant pathology methods. Boca Raton, FL: CRC Press.
43. Maniatis *et al.,* 1985. Molecular Cloning: A Laboratory Manual: DNA Cloning. New York: Cold Spring Harbor.
44. Gait 1984. Oligonucleotide Synthesis-A Practical Approach. Washington, D.C.: IRL Press.
45. Hames *et al.,* 1984. Nucleic Acid Hybridization: A Practical Approach. Washington D.C.: IRL Press.
46. Watson *et al.,* 1992. Recombinant DNA. New York: Scientific American Books.
47. Szewczyk *et al.,* 2006. "Fusion PCR and gene targeting in *Aspergillus nidulans*." Nat Protoc, 1: 3111-3121.
48. Ho *et al.,* 1989. "Site-directed mutagenesis by overlap extension using the polymerase chain reaction." Gene, 77: 51-59.
49. Fuhrmann *et al.,* 1999. "A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii*." Plant J, 19: 353-361.
50. Mandecki *et al.,* 1988. "FokI method of gene synthesis." Gene, 68: 101–107.
51. Stemmer 1995. "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides." Gene, 164: 49–53.
52. Gao *et al.,* 2003. "Thermodynamically balanced inside-out (TBIO) PCR-based gene synthesis: a novel method of primer design for high-fidelity assembly of longer gene sequences." Nucleic Acids Res, 31: e143.
53. Young *et al.,* 2004. "Two-step total gene synthesis method." Nucleic Acids Res, 32: e59.
54. van Der Krol *et al.,* 1999. "Developmental and wound-, cold-, desiccation-, ultraviolet-B-stress-induced modulations in the expression of the petunia zinc finger transcription factor gene ZPT2-2." Plant Physiol, 121: 1153-62.
55. Shinmyo *et al.,* 1998. "Metabolic engineering of cultured tobacco cells." Biotechnol Bioeng, 58: 329-32.
56. Sohal *et al.,* 1999. "The promoter of a *Brassica napus* lipid transfer protein gene is active in a range of tissues and stimulated by light and viral infection in transgenic Arabidopsis." Plant Mol Biol, 41: 75-87.
57. Cormack *et al.,* 2002. "Leucine zipper-containing WRKY proteins widen the spectrum of immediate early elicitor-induced WRKY transcription factors in parsley." Biochim Biophys Acta, 1576: 92-100.
58. Eulgem *et al.,* 1999. "Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors." EMBO (Eur Mol Biol Organ) J, 18: 4689-99.
59. Lebel *et al.,* 1998. "Functional analysis of regulatory sequences controlling PR-1 gene expression in *Arabidopsis*." Plant J, 16: 223-33.
60. Ngai *et al.,* 1997. "Light-induced transcriptional repression of the pea AS1 gene: identification of cis-elements and transfactors." Plant J, 12: 1021-34.

61. Kucho *et al.,* 1999. "CO(2)-responsive transcriptional regulation of CAH1 encoding carbonic anhydrase is mediated by enhancer and silencer regions in *Chlamydomonas reinhardtii*." Plant Physiol, 121: 1329-38.
62. Kucho *et al.,* 2003. "Cis-acting elements and DNA-binding proteins involved in CO2-responsive transcriptional activation of Cah1 encoding a periplasmic carbonic anhydrase in *Chlamydomonas reinhardtii*." Plant Physiol, 133: 783-93.
63. Chen *et al.,* 1996. "The promoter of a H2O2-inducible, *Arabidopsis* glutathione S-transferase gene contains closely linked OBF- and OBP1-binding sites." Plant J, 10: 955-66.
64. Chen *et al.,* 1999. "The auxin, hydrogen peroxide and salicylic acid induced expression of the *Arabidopsis* GST6 promoter is mediated in part by an ocs element." Plant J, 19: 667-77.
65. Lu *et al.,* 1998. "Sugar response sequence in the promoter of a rice alpha-amylase gene serves as a transcriptional enhancer." J Biol Chem, 273: 10120-31.
66. Leubner-Metzger *et al.,* 1998. "Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I beta-1,3-glucanase during tobacco seed germination." Plant Mol Biol, 38: 785-95.
67. Hudspeth *et al.,* 1992. "Expression of maize phosphoenolpyruvate carboxylase in transgenic tobacco : Effects on biochemistry and physiology." Plant Physiol, 98: 458-464.
68. de Framond 1991. "A metallothionein-like gene from maize (*Zea mays*). Cloning and characterization." FEBS Lett, 290: 103-6.
69. Hudspeth *et al.,* 1996. "Characterization and expression of metallothionein-like genes in cotton." Plant Mol Biol, 31: 701-5.
70. Herrera-Estrella *et al.,* 1983. "Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector." Nature, 303: 209-213.
71. Pathirana *et al.,* 1997. "Analyses of phosphoenolpyruvate carboxylase gene structure and expression in alfalfa nodules." Plant J, 12: 293-304.
72. Yang *et al.,* 2002. "Isolation and characterization of the orchid cytokinin oxidase DSCKX1 promoter." J Exp Bot, 53: 1899-1907.
73. Moon *et al.,* 2004. "Developmental regulation of peach ACC oxidase promoter–GUS fusions in transgenic tomato fruits." J Exp Bot, 55: 1519-1528.
74. Avsian-Kretchmer *et al.,* 2004. "The salt-stress signal transduction pathway that activates the gpx1 promoter is mediated by intracellular H2O2, different from the pathway induced by extracellular H2O2." Plant Physiol, 135: 1685-96.
75. Wu *et al.,* 2007. "Functional analysis of a cotton glucuronosyltransferase promoter in transgenic tobaccos." Cell Res, 17: 174–183.
76. Shelp *et al.,* 1999. "Metabolism and functions of gamma-aminobutyric acid." Trends Plant Sci, 41: 446-452.
77. Baum *et al.,* 1993. "A plant glutamate decarboxylase containing a calmodulin binding domain. Cloning, sequence, and functional analysis." J Biol Chem, 268: 19610-19617.
78. Gallego *et al.,* 1995. "A role for glutamate decarboxylase during tomato ripening: the characterisation of a cDNA encoding a putative glutamate decarboxylase with a calmodulin-binding site." Plant Mol Biol, 27: 1143-1151.

79. Yun *et al.,* 1998. "Cloning and characterization of tobacco cDNA encoding calcium/calmodulin-dependent glutamate decarboxylase." Mol Cell, 8: 125-129.
80. Oh *et al.,* 2005. "Cloning and characterization of a rice cDNA encoding glutamate decarboxylase." J Biochem Mol Biol, 38: 595-601.
81. Chiu *et al.,* 2002. "Phylogenetic and expression analysis of the glutamate-receptor-like gene family in *Arabidopsis thaliana*." Mol Biol Evol, 19: 1066-1082.
82. Kim *et al.,* 2001. "Overexpression of the AtGluR2 gene encoding an *Arabidopsis* homolog of mammalian glutamate receptors impairs calcium utilization and sensitivity to ionic stress in transgenic plants." Plant Cell Physiol, 42: 74-84.
83. Meyerhoff *et al.,* 2005. "AtGLR3.4, a glutamate receptor channel-like gene is sensitive to touch and cold." Planta, 222: 418-27.
84. Maruyama-Nakashita *et al.,* 2004. "Regulation of high-affinity sulphate transporters in plants towards systematic analysis of sulphur signalling and regulation." J Exp Bot, 55: 1843-1849.
85. Yoshimoto *et al.,* 2003. "Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis*." Plant Physiol, 131: 1511-1517.
86. Awazuhara *et al.,* 2005. "The function of SULTR2;1 sulfate transporter during seed development in *Arabidopsis thaliana*." Physiol Plant, 125: 95-105.
87. Kataoka *et al.,* 2004. "Root-to-shoot transport of sulfate in Arabidopsis: Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature." Plant Physiol, 136: 4198-4204.
88. An *et al.,* 1985. "New cloning vehicles for transformation of higher plants." EMBO (Eur Mol Biol Organ) J, 4: 277-284.
89. Gruber *et al.,* 1993. Vectors for plant transformation. *In* Glick BR & JE Thompson, editors. Methods in Plant Molecular Biology and Biotechnology 89-119. Baco Raton, Fla.: CRC Press.
90. Ausubel *et al.,* 1995. Current Protocols in Molecular Biology. New York: Greene Publishing and Wiley-Interscience.
91. Newman *et al.,* 1993. "DST sequences, highly conserved among plant SAUR genes, target reporter transcripts for rapid decay in tobacco." Plant Cell, 5: 701-14.
92. Ohme-Takagi *et al.,* 1993. "The effect of sequences with high AU content on mRNA stability in tobacco." Proc Natl Acad Sci U S A, 90: 11811-5.
93. von Heijne 1986. "Mitochondrial targeting sequences may form amphiphilic helices." EMBO (Eur Mol Biol Organ) J, 5: 1335–1342.
94. Swinkels *et al.,* 1991. "A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase." EMBO (Eur Mol Biol Organ) J, 10: 3255-62.
95. Rusch *et al.,* 1995. "Protein transport via amino-terminal targeting sequences: Common themes in diverse systems." Mol Membr Biol, 12: 295-307.
96. Soll *et al.,* 1998. "Protein translocation into and across the chloroplastic envelope membranes." Plant Mol Biol, 38: 191–207.
97. Gould *et al.,* 1988. "Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins." J Cell Biol, 107: 897-905.

98. Gould *et al.,* 1989. "A conserved tripeptide sorts proteins to peroxisomes." J Cell Biol, 108: 1657-64.
99. McCammon *et al.,* 1994. "An internal region of the peroxisomal membrane protein PMP47 is essential for sorting to peroxisomes." J Cell Biol, 124: 915-25.
100. Cokol *et al.,* 2000. "Finding nuclear localization signals." EMBO Rep, 1: 411-5.
101. Helenius *et al.,* 2001. "Intracellular functions of N-linked glycans." Science, 291: 2364-9.
102. Emanuelsson *et al.,* 2007. "Locating proteins in the cell using TargetP, SignalP and related tools." Nat Protoc, 2: 953-971.
103. Emanuelsson *et al.,* 2000. "Predicting subcellular localization of proteins based on their N-terminal amino acid sequence." J Mol Biol, 300: 1005-1016.
104. Bannai *et al.,* 2002. "Extensive feature detection of N-terminal protein sorting signals." Bioinformatics, 18: 298-305.
105. Bendtsen *et al.,* 2004. "Improved prediction of signal peptides: SignalP 3.0." J Mol Biol, 340: 783-95.
106. Hiller *et al.,* 2004. "PrediSi: prediction of signal peptides and their cleavage positions." Nucleic Acids Res, 32: W375-9.
107. Bhasin *et al.,* 2004. "ESLpred: SVM-based method for subcellular localization of eukaryotic proteins using dipeptide composition and PSI-BLAST." Nucleic Acids Res, 32: W414-9.
108. Garg *et al.,* 2005. "Support vector machine-based method for subcellular localization of human proteins using amino acid compositions, their order, and similarity search." J Biol Chem, 280: 14427-32.
109. Bhasin *et al.,* 2005. "PSLpred: prediction of subcellular localization of bacterial proteins." Bioinformatics, 21: 2522-4.
110. Hoglund *et al.,* 2006. "MultiLoc: prediction of protein subcellular localization using N-terminal targeting sequences, sequence motifs and amino acid composition." Bioinformatics, 22: 1158-65.
111. Shatkay *et al.,* 2007. "SherLoc: high-accuracy prediction of protein subcellular localization by integrating text and protein sequence data." Bioinformatics, 23: 1410-7.
112. Emanuelsson *et al.,* 1999. "ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites." Protein Sci, 8: 978-984.
113. Claros *et al.,* 1996. "Computational method to predict mitochondrially imported proteins and their targeting sequences." Eur J Biochem, 241: 779-86.
114. Small *et al.,* 2004. "Predotar: A tool for rapidly screening proteomes for N-terminal targeting sequences." Proteomics, 4: 1581-1590.
115. Kelley *et al.,* 2000. "Enhanced genome annotation using structural profiles in the program 3D-PSSM." J Mol Biol, 299: 499-520.
116. Bülow *et al.,* 1991. "Multienzyme systems obtained by gene fusion." Trends Biotechnol, 9: 226-231.
117. Seo *et al.,* 2000. "Characterization of a bifunctional enzyme fusion of trehalose-6-phosphate synthetase and trehalose-6-phosphate phosphatase of *Escherichia coli*." Appl Environ Microbiol, 66: 2484-2490.
118. Honjoh *et al.,* 2009. "Enhancement of menadione stress tolerance in yeast by accumulation of hypotaurine and taurine: co-expression of cDNA clones, from *Cyprinus*

carpio, for cysteine dioxygenase and cysteine sulfinate decarboxylase in *Saccharomyces cerevisiae*." Amino Acids, [Epub ahead of print].
119. Shahin 1985. "Totipotency of tomato protoplasts." Theor Appl Genet, 69: 235-240.
120. Fromm *et al.,* 1985. "Expression of genes transferred into monocot and dicot plant cells by electroporation." Proc Natl Acad Sci U S A, 82: 5824-5828.
121. Fromm *et al.,* 1986. "Stable transformation of maize after gene transfer by electroporation." Nature, 319: 791-3.
122. Riggs *et al.,* 1986. "Stable transformation of tobacco by electroporation: evidence for plasmid concatenation." Proc Natl Acad Sci U S A, 83: 5602-5606.
123. D'Halluin *et al.,* 1992. "Transgenic maize plants by tissue electroporation." Plant Cell, 4: 1495-1505.
124. Laursen *et al.,* 1994. "Production of fertile transgenic maize by electroporation of suspension culture cells " Plant Mol Biol, 24: 51-61
125. Crossway *et al.,* 1986. "Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts." Mol Gen Genet, 202: 179-185.
126. Griesbach 1983. "Protoplast microinjection." Plant Mol Biol Report, 1: 32-37.
127. Sporlein *et al.,* 1991. "Lipofectin: direct gene transfer to higher plants using cationic liposomes." Theor Appl Genet, 83: 1-5.
128. Ohgawara *et al.,* 1983. "Uptake of liposome-encapsulated plasmid DNA by plant protoplasts and molecular fate of foreign DNA " Protoplasma, 116: 145-148.
129. Deshayes *et al.,* 1985. "Liposome-mediated transformation of tobacco mesophyll protoplasts by an *Escherichia coli* plasmid." EMBO (Eur Mol Biol Organ) J, 4: 2731-7.
130. Christou *et al.,* 1987. "Stable transformation of soybean by electroporation and root formation from transformed callus." Proc Natl Acad Sci U S A, 84: 3962-3966.
131. Horsch *et al.,* 1985. "A simple and general method for transferring genes into plants." Science, 227: 1229-1231.
132. Paszkowski *et al.,* 1984. "Direct gene transfer to plants." Embo J, 3: 2717-2722.
133. Hooykaas-Van Slogteren *et al.,* 1984. "Expression of Ti plasmid genes in monocotyledonous plants infected with *Agrobacterium tumefaciens*." Nature, 311: 763-764.
134. Rogers 1986. "Gene transfer in plants: Production of transformed plants using Ti-plasmid vectors." Methods Enzymol, 118: 627-640.
135. Bevan *et al.,* 1982. "T-DNA of the *Agrobacterium* Ti and Ri plasmids." Annu Rev Genet, 16: 357-384.
136. Klein *et al.,* 1988. "Transfer of foreign genes into intact maize cells with high-velocity microprojectiles." Proc Natl Acad Sci U S A, 85: 4305-4309.
137. Klein *et al.,* 1988. "Factors influencing gene delivery into *Zea mays* cells by high-velocity microprojectiles." Biotechnology, 6: 559-563.
138. McCabe *et al.,* 1988. "Stable transformation of soybean (*Glycine max*) by particle acceleration." Biotechnology, 6: 923-926.
139. Sanford *et al.,* 1993. Optimizing the biolistic process for different biological application. *In* Wu R, editor. The Methods in Enzymology 483-509. Orlando: Academic Press.
140. Freeman *et al.,* 1984. "A comparison of methods for plasmid delivery into plant protoplasts." Plant Cell Physiol, 25: 1353-1365.

141. Frame *et al.,* 1994. "Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation." Plant J, 6: 941–948.
142. Thompson *et al.,* 1995. "Maize transformation utilizing silicon carbide whiskers: a review." Euphytica, 85: 75–80.
143. Guo *et al.,* 1995. "Laser-mediated gene transfer in rice." Physiol Plant, 93: 19–24.
144. Badr *et al.,* 2005. " Production of fertile transgenic wheat plants by laser micropuncture." Photochem Photobiol Sci, 4: 803-807.
145. Bao *et al.,* 1997. " Transfection of a reporter plasmid into cultured cells by sonoporation in vitro." Ultrasound in Medicine and Biology, 23: 953-959.
146. Finer *et al.,* 2000. "Use of *Agrobacterium* expressing green fluorescent protein to evaluate colonization of sonication-assisted *Agrobacterium*-mediated transformation-treated soybean cotyledons." Lett Appl Microbiol, 30: 406-10.
147. Amoah *et al.,* 2001. "Factors influencing *Agrobacterium*-mediated transient expression of uidA in wheat inflorescence tissue." J Exp Bot, 52: 1135-42.
148. Krens *et al.,* 1982. "In Vitro transformation of plant protoplasts with Ti-plasmid DNA." Nature, 296: 72-74.
149. Bechtold *et al.,* 1998. "In planta *Agrobacterium*-mediated transformation of adult Arabidopsis thaliana plants by vacuum infiltration." Methods Mol Biol, 82: 259-66.
150. Broothaerts *et al.,* 2005. "Gene transfer to plants by diverse species of bacteria." Nature, 433: 629-633.
151. Kirk *et al.,* 1993. Concise Encyclopedia of Chemical Technology: John Wiley & Sons,.
152. Mosbach *et al.,* 1983. " Formation of proinsulin by immobilized *Bacillus subtilis*." Nature, 302: 543-545.
153. Chan *et al.,* 1974. "Structural uniqueness of lactose operator." Nature, 252: 205-209.
154. Goeddel *et al.,* 1980. "Synthesis of human fibroblast interferon by *E. coli* " Nucleic Acids Res, 8: 4057-4074.
155. Sherman *et al.,* 1982. Methods in Yeast Genetics. New York: Cold Spring Harbor Laboratory.
156. Sherman 1991. Getting started with yeast. *In* Guthrie C & GR Fink, editors. Methods in Enzymology, Guide to Yeast Genetics and Molecular Biology 3-21. New York: Acad. Press.
157. Andersen *et al.,* 1984. "Synthesis and anticonvulsant properties of some 2-Aminoethanesulfonic acid (Taurine) derivatives." J Pharm Sci, 73: 106-108.
158. Herdeis *et al.,* 1999. United States Patent No. 5889183.
159. Tserng *et al.,* 1977. "An improved procedure for the synthesis of glycine and taurine conjugates of bile acids." J Lipid Res, 18: 404-407.
160. Fong *et al.,* 1992. United States Patent No. 5128419.
161. Seeberger *et al.,* 2007. "A new strategy for the synthesis of taurine derivatives using the 'safety-catch' principle for the protection of sulfonic acids." Org Biomol Chem, 5: 132-138.
162. Suzuki *et al.,* 2006. "Fabrication of TiO2 using L-lysine-based organogelators as organic templates: control of the nanostructures." Chem Commun377-379.

163. Mikhalenko *et al.,* 2004. "Phthalocyanines and related compounds: XXXVIII. Synthesis of symmetric taurine- and choline-substituted phthalocyanines." Russ J Gen Chem, 74: 1775-1800.
164. Capone *et al.,* 2007. "Designing nanosensors based on charged derivatives of Gramicidin A." J Am Chem Soc, 129: 9737-9745.
165. Gupta *et al.,* 2005. "Taurine analogues; A new class of therapeutics: Retrospect and prospects " Curr Med Chem, 12: 2021-2039.
166. Johnson 2008. "Update on neuropharmacological treatments for alcoholism: Scientific basis and clinical findings." Biochem Pharmacol, 75: 34-56.
167. Tambour *et al.,* 2007. "Preclinical and clinical pharmacology of alcohol dependence." Fundam Clin Pharmacol, 21: 9-28.
168. Joung *et al.,* 2005. "Anticoagulant supramolecular-structured polymers: Synthesis and anticoagulant activity of taurine-conjugated carboxyethylester-polyrotaxanes." Sci Technol Adv Mater, 6: 484-490.
169. Özmeriç *et al.,* 2000. "Chitosan film enriched with an antioxidant agent, taurine, in fenestration defects." J Biomed Mater Res A, 51: 500 - 503.
170. Degim *et al.,* 2002. "An investigation on skin wound healing in mice with a taurinechitosan gel formulation." Amino Acids, 22: 187-198.
171. Matsusaki *et al.,* 2002. "Novel functional biodegradable polymer: Synthesis and anticoagulant activity of poly(γ-Glutamic Acid)sulfonate (γ-PGA-sulfonate)." Bioconjugate Chem, 13: 23-28.
172. Meinkoth *et al.,* 1984. "Hybridization of nucleic acids immobilized on solid supports." Anal Biochem, 138: 267-284.
173. Tijssen 1993. Overview of principles of hybridization and the strategy of nucleic acid probe assays. Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes: Part I. New York: Elsevier.
174. Smith *et al.,* 1981. "Comparison of biosequences." Adv Appl Math, 2: 482–489.
175. Needleman *et al.,* 1970. "A general method applicable to the search for similarities in the amino acid sequence of two proteins." J Mol Biol, 48: 443-453.
176. Pearson *et al.,* 1988. "Improved tools for biological sequence comparison." Proc Natl Acad Sci U S A, 85: 2444–2448.
177. Higgins *et al.,* 1989. "Fast and sensitive multiple sequence alignments on a microcomputer." Comput Appl Biosci, 5: 151-153.
178. Higgins *et al.,* 1988. "CLUSTAL: a package for performing multiple sequence alignment on a microcomputer." Gene, 73: 237-244.
179. Higgins *et al.,* 1992. "CLUSTAL V: improved software for multiple sequence alignment." Comput Appl Biosci, 8: 189-191.
180. Feng *et al.,* 1987. "Progressive sequence alignment as a prerequisite to correct phylogenetic trees." J Mol Evol, 25: 351-360.
181. Henikoff *et al.,* 1989. "Amino acid substitution matrices from protein blocks " Proc Natl Acad Sci U S A, 89: 10915-10919.
182. Altschul *et al.,* 1997. "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res, 25: 3389-3402.

183. Wootton *et al.,* 1993. "Statistics of local complexity in amino acid sequences and sequence databases." Comput Chem, 17: 149-163.
184. Wootton *et al.,* 1996. "Analysis of compositionally biased regions in sequence databases." Methods Enzymol, 266: 554-571.
185. Claverie *et al.,* 1993. "Information enhancement methods for large scale sequence analysis." Comput Chem, 17: 191–201.
186. Myers *et al.,* 1988. "Optimal alignments in linear-space." Comput Appl Biol Sci, 4: 11-17.

[168] All patents, patent applications, and references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Development of a transgenic plant that constitutively expresses COD using fusion PCR

[169] Step 1: Make a DNA construct that contains an *AtTUB5* promoter with a *CDO* gene and a NOS terminator in the following manner.

[170] Step 1a. Use PCR to amplify the *AtTUB5* promoter (-1851 to -1 bps) with a short overlap for the 5′ end of CDO at the 3′ end of the promoter using 500 ng of genomic DNA isolated from an *Arabidopsis thaliana* Col-0. Add 300 nM of the following primers: 5′KpnTub5prom (5′-ttttggtacccacatttgcaaaatgatgaatg-3′; SEQ ID NO:27) and Tub5CDO (5′-catgacttcagtctgctccatccaatctggttaccgcattg-3′; SEQ ID NO:28). Run the fusion PCR as described by Szewczyk et al. (45).

[171] Step 1b: Use PCR to amplify the *CDO* gene from 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: 5′CDO (5′-atggagcagactgaagtcatg-3′; SEQ ID NO:29) and 3′CDO (5′-tcagttattctcctgcgagac-3′; SEQ ID NO:30). Run the fusion PCR as described by Szewczyk et al. (45).

[172] Step 1c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of CDO at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers CDONOS (5′-gtctcgcaggagaataactgagctaccgagctcgaatttcc-3′; SEQ ID NO:31) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[173] Step 1d: Combine the amplified fragments from Example 3: steps 1a, 1b, and 1c and 300 nM of the following primers GP2 (5′-ttttggtaccgtttacatatggagatgatgtc-3′; SEQ ID NO:33) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[174] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence. Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[175] Step 1f. Transform the ligated vector containing the DNA construct by electroporation into *E. coli*. Select for kanamycin (50 μg/ml) resistance on LB plates. Confirm the presence of the DNA constructs in the selected colonies by PCR analysis with the GP2 and GP5 primers using the following program: 96°C for 3 min followed by 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 5 min, and 72°C for 3 min. Grow a colony that contains the proper DNA construct overnight at 37°C in 6 ml LB plus spectinomycin (100 μg/ml) or streptomycin (200 μg/ml). Isolate the plasmid DNA that contains the DNA construct by Wizard Plus SV Minipreps DNA Purification System (Promega Corporation, Madison, WI, USA). Sequence the DNA insert to confirm its identity and the fidelity of the DNA construct.

[176] Step 2: Transform *Agrobacterium tumefaciens*

[177] Independently transform the vector construct into electrocompetent *Agrobacterium tumefaciens* EHA105, as described by the Green Lab Protocol (http://www.bch.msu.edu/pamgreen/green.htm). Select positive transformants using Terrific Broth plus kanamycin (50 μg/ml) on 1% agar plates. Confirm *Agrobacterium* colonies by PCR using the following primers: GP2 and GP5. Run the following PCR reaction: 96°C for 5 min followed by 20 cycles of 94°C for 45 seconds, 60°C for 30 seconds, 70°C for 5 min, and 72°C for 3 min.

[178] Step 3: Transform plant, *Arabidopsis thaliana*

[179] Step 3a: Sow *Arabidopsis* (L.) Heynh. ecotype Columbia (Col-0) seeds in 248 cm^2 plastic pots with moistened soil (Promix HP, Premier Horticulture Inc., Redhill, PA, Canada). Grow plants at 20-21°C, with 60-70% relative humidity, under cool white fluorescent lights (140 μmol m^{-2} s^{-1}) with a 16 h light/8 h dark cycle. Water plants as needed by subirrigation. After two weeks, transfer five individual plants to smaller pots (72 cm^2) for use in the transformation protocol. Grow the plants until the first floral buds and flowers form (2-3 additional weeks).

[180] Step 3b: Grow *Agrobacterium,* the construct to be transformed, in 500 ml of Terrific Broth plus kanamycin (50 μg/ml) for 2 days at 29°C. Collect cells by centrifugation at 6000 rpm for 15 minutes, and resuspend cells in 5% sucrose plus 0.05% surfactant (Silwet L-77, Lehle Seeds, Round Rock, TX, USA) solution.

[181] Step 3c: Transform plants by the floral dip transformation (144). Keep the plants in sealed containers to maintain high humidity for 16 to 24 h and maintain plants as described in step 4a above. At 8 to 10 weeks, dry the plants, collect the seeds, and select for the marker in each line. Select for kanamycin resistance for the *AtTUB5::CDO* constructs in pCAMBIA2300 by incubating seeds on plates containing 4.418g/L Murashige and Skoog Salt and Vitamin Mixture (MS medium, Life Technologies, Grand Island, NY, USA) plus kanamycin (50 μg/ml) and 0.8% (wt vol) Phytagar. Collect and transfer positively selected plants into pots containing soil and grow for 5 to 6 weeks.

Allow the plants to self-pollinate. Collect the seeds and repeat the selection process until homozygotes are identified.

EXAMPLE 2

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO and SAD using fusion PCR

[182] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a *CDO* gene and a NOS terminator in the following manner.

[183] Step 1a: Use PCR to amplify the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5′ end of CDO at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtPHYB (5′-caatgcctaataatgtctagc-3′; SEQ ID NO:35) and AtPHYBCDO (5′-catgacttcagtctgctccatgccgtttgattttgaatttgag-3′; SEQ ID NO:36). Run the fusion PCR as described by Szewczyk et al. (45).

[184] Step 1b: Use the CDO gene that was amplified in Example 1: Step 1b.

[185] Step 1c: Use the NOS terminator with a short overlap for the 3′ end of CDO at the 5′ end of the NOS terminator that was amplified in Example 1: Step 1c.

[186] Step 1d: Combine the PCR fragments (Example 2: 1a, 1b, and 1c) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[187] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and XbaI.

[188] Step 2: Make a DNA construct that contains an *AtPHYB* promoter with a *SAD* gene and a NOS terminator to in the following manner.

[189] Step 2a: Use PCR to amplify the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5′ end of SAD at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtPHYB (5′-caatgcctaataatgtctagc -3′; SEQ ID NO:35) and AtPHYBSAD (5′-cagcttcccatcagactcgtccatgccgtttgattttgaatttgag-3′; SEQ ID NO:38). Run the fusion PCR as described by Szewczyk et al. (45).

[190] Step 2b: Use PCR to amplify the *SAD* gene from 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: 5′SAD (5′-atggacgagtctgatgggaagctg-3′; SEQ ID NO:39) and 3′SAD (5′-tcatagatccttcccgagtttc-3′; SEQ ID NO:40). Run the fusion PCR as described by Szewczyk et al. (45).

[191] Step 2c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of SAD at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers SADNOS (5′-gaaactcgggaaggatctatgagctaccgagctcgaatttcc-3′; SEQ ID NO:41) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[192] Step 2d: Combine the PCR fragments (Example 2: 2a, 2b, and 2c) and 300 nM of the following primers IP2 (5′-aaaaatctagaattcttgaattacgattgtac-3′; SEQ ID NO:42) and IP5 (5′-ttttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[193] Step 2e: Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify the plasmid that contains the DNA construct to confirm its identity and the fidelity of the sequence as described in Example

1: step 1e.purify. Digest the plasmid with XbaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XbaI and SalI.

[194] Step 3: Ligate the *AtPHYB* promoter-CDO-NOS terminator construct upstream of the *AtPHYB* promoter-SAD-NOS terminator construct into a plant expression vector.

[195] Step 3a. Digest the pCambia2300-*AtPHYB* promoter-CDO-NOS terminator clone (from Example 4: Step 1e) with XmaI and XbaI, isolate DNA insert and ligate it into the vector pCambia2300-*AtPHYB* promoter-SAD-NOS terminator (from Example 4: Step 2e) that has been predigested with XmaI and XbaI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[196] Step 4: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[197] Step 5: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 3

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO and GAD using fusion PCR

[198] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a CDO gene and a NOS terminator in the following manner.

[199] Step 1a: Use the *AtPHYB* promoter with a short overlap for the 5′ end of CDO at the 3′ end that was amplified in Example 2: Step 1a

[200] Step 1b: Use the *CDO* gene that was amplified in Example 1: Step 1b.

[201] Step 1c: Use the NOS terminator with a short overlap for the 3′ end of CDO at the 5′ end of the NOS terminator that was amplified in Example 1: Step 1c.

[202] Step 1d: Combine the PCR fragments (Example 3: 1a, 1b, and 1c), run the PCR and the clone the amplified fragment as described in Example 2: Step 1d.

[203] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and XbaI.

[204] Step 2: Make a DNA construct that contains an *AtPHYB* promoter with a GAD gene and a NOS terminator to in the following manner.

[205] Step 2a: Use PCR to amplify the *AtPHYB* promoter (-1960 to -1 bps) with a short overlap for the 5′ end of GAD at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtPHYB (5′-caatgcctaataatgtctagc -3′; SEQ ID NO:35) and AtPHYBGAD (5′-cgttacttgcttcttatccatgccgtttgattttgaatttgag-3′; SEQ ID NO:44). Run the fusion PCR as described by Szewczyk et al. (45).

[206] Step 2b: Use PCR to amplify the GAD gene from 500 ng of DNA from E. coli strain K12. Add 300 nM of the following primers: 5′GAD (5′-atggataagaagcaagtaacg-3′; SEQ ID NO:45) and 3′GAD (5′-tcaggtatgtttaaagctgttc-3′; SEQ ID NO:46). Run the fusion PCR as described by Szewczyk et al. (45).

[207] Step 2c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of GAD at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers GADNOS (5′-gaacagctttaaacatacctgagctaccgagctcgaatttcc-3′; SEQ ID NO:47) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[208] Step 2d: Combine the PCR fragments (Example 3: 2a, 2b, and 2c) and 300 nM of the following primers IP2 (5′-aaaaatctagaattcttgaattacgattgtacc-3′; SEQ ID NO:42) and IP5 (5′-ttttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[209] Step 2e: Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify the plasmid that contains the DNA construct to confirm its identity and the fidelity of the sequence as described in Example 1: step 1e.purify. Digest the plasmid with XbaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XbaI and SalI.

[210] Step 3: Ligate the *AtPHYB* promoter-CDO-NOS terminator construct upstream of the *AtPHYB* promoter-GAD-NOS terminator construct into a plant expression vector.

[211] Step 3a. Digest the pCambia2300-*AtPHYB* promoter-CDO-NOS terminator clone (from Example 4: Step 1e) with XmaI and XbaI, isolate DNA insert and ligate it into the vector pCambia2300-*AtPHYB* promoter-GAD-NOS terminator (from Example 4: Step 2e) that has been predigested with XmaI and XbaI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[212] Step 4: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[213] Step 5: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 4

Development of a transgenic plant that non-constitutively expresses (AtGAD1 promoter) ADO using fusion PCR

[214] Step 1: Make a DNA construct that contains an *AtGAD1* (Locus ID# At5g17330) promoter with an *ADO* gene and a NOS terminator in the following manner.

[215] Step 1a: Use PCR to amplify the *AtGAD1* promoter (-1732 to -1 bps) with a short overlap for the 5′ end of ADO at the 3′ end of the promoter using 500 ng of genomic DNA isolated from an *Arabidopsis thaliana* Col-0. Add 300 nM of the following primers: 5′AtGAD1 (5′-accaaaggataccctgatttg-3′; SEQ ID NO:48) and AtGAD1ADO (5′-gattttctggactgtggaagtcatcacggagatgagagagagag-3′; SEQ ID NO:49). Run the fusion PCR as described by Szewczyk et al. (45).

[216] Step 1b: Use PCR to amplify the *ADO* gene from 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: 5′ADO (5′-atgacttccacagtccagaaaatc-3′; SEQ ID NO:50) and 3′ADO (5′-tcagagggtcactttaggc-3′; SEQ ID NO:51). Run the fusion PCR as described by Szewczyk et al. (45).

[217] Step 1c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of ADO at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers ADONOS (5′-gcctaaagtgaccctctgagctaccgagctcgaatttcc-3′; SEQ ID NO:52) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[218] Step 1d: Combine the amplified fragments (Example 4: steps 1a, 1b, and 1c) and 300 nM of the following primers JP2 (5′-aaaaaggtaccgatatttgagcaaaactgtgg-3′; SEQ ID NO:30) and GP5 (5′-ttttttTCTAGAgatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[219] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[220] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[221] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[222] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 5

Development of a transgenic plant that non-constitutively expresses (AtGAD2 promoter) TPAT using fusion PCR

[223] Step 1: Make a DNA construct that contains an *AtGAD2* (Locus ID# At1g65960) promoter with a *TPAT* gene and a NOS terminator in the following manner.

[224] Step 1a: Use PCR to amplify the *AtGAD2* promoter (-1714 to -1 bps) with a short overlap for the 5′ end of *TPAT* at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtGAD2 (5′-tcttaccttgtcctgcaacgag-3′; SEQ ID NO:54) and AtGAD2TPAT (5′-cattgaaattgccgtccatctttgtttctgtttagtgaaag-3′; SEQ ID NO:55). Run the fusion PCR as described by Szewczyk et al. (45).

[225] Step 1b: Use PCR to amplify the *TPAT* gene from 500 ng of DNA from *Roseobacter denitrificans* strain. Add 300 nM of the following primers: 5′TPAT (5′-atggacggcaatttcaatg-3′; SEQ ID NO:56) and 3′TPAT (5′-ttagccgaaaacgcgcgacag-3′; SEQ ID NO:57). Run the fusion PCR as described by Szewczyk et al. (45).

[226] Step 1c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of TPAT at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers TPATNOS (5′-ctgtcgcgcgttttcggctaagctaccgagctcgaatttcc-3′; SEQ ID NO:58) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[227] Step 1d: Combine the amplified fragments (Example 5: steps 1a, 1b, and 1c) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[228] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[229] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[230] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[231] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 6

Development of a transgenic plant that non-constitutively expresses (AtGAD2 promoter) TPAT and SA using fusion PCR

[232] Step 1: Make a DNA construct that contains an *AtGAD2* (Locus ID#) promoter with a *TPAT* gene and a NOS terminator in the following manner.

[233] Step 1a: Use the *AtGAD2* promoter that was amplified in Example 5: Step 1b

[234] Step 1b: Use the *TPAT* gene that was amplified in Example 5: Step 1b.

[235] Step 1c: Use the NOS terminator with a short overlap for the 3′ end of *TPAT* at the 5′ end of the NOS terminator that was amplified in Example 5: Step 1c.

[236] Step 1d: Combine the PCR fragments (Example 5: 1a, 1b, and 1c), run the PCR and the clone the amplified fragment as described in Example 5: Step 1d.

[237] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[238] Step 2: Make a DNA construct that contains an *AtGAD2* promoter with a *SA* gene and a NOS terminator to in the following manner.

[239] Step 2a: Use PCR to amplify the *AtGAD2* promoter (-1960 to -1 bps) with a short overlap for the 5′ end of SA at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtGAD2 (5′-

cttaccttgtcctgcaacgag-3′; SEQ ID NO:54) and AtGAD2SA (5′-cttcagtggtcattttcatctttgtttctgtttagtgaaag-3′; SEQ ID NO:60). Run the fusion PCR as described by Szewczyk et al. (45).

[240] Step 2b: Use PCR to amplify the *SA* gene from 500 ng of DNA from Roseobacter denitrificans. Add 300 nM of the following primers: 5′SA (5′-atgaaaatgaccactgaag-3′; SEQ ID NO:61) and 3′SA (5′-tcagacagtctgtggacgc-3′; SEQ ID NO:62). Run the fusion PCR as described by Szewczyk et al. (45).

[241] Step 2c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of SA at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers SANOS (5′-gcgtccacagactgtctgagctaccgagctcgaatttcc-3′; SEQ ID NO:63) and 3′NOS ; 5′-cacgacgttgtaaaacgacggc-3′ SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[242] Step 2d: Combine the PCR fragments (Example 6: 2a, 2b, and 2c) and 300 nM of the following primers LP2 (5′-tttttctagagaacgagcttcaacgtagcc-3′; SEQ ID NO:64) and LP5 (5′-aaaaaaagcttgatctagtaacatagatgacac-3′; SEQ ID NO:65). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[243] Step 2e: Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify the plasmid that contains the DNA construct to confirm its identity and the fidelity of the sequence as described in Example 1: step 1e.purify. Digest the plasmid with XbaI and HindIII, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XbaI and HindIII.

[244] Step 3: Ligate the *AtGAD2* promoter-TPAT-NOS terminator construct upstream of the *AtGAD2* promoter-SA-NOS terminator construct into a plant expression vector.

[245] Step 3a. Digest the pCambia2300-*AtGAD2* promoter-TPAT-NOS terminator clone (from Example 4: Step 1e) with Acc65I and XbaI, isolate DNA insert and ligate it into the

vector pCambia2300-*AtGAD2* promoter-SA-NOS terminator (from Example 6: Step 2e) that has been predigested with Acc65I and XbaI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[246] Step 4: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[247] Step 5: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 7

Development of a transgenic plant that that non-constitutively expresses (AtGLR1.1 promoter) ssTDeHase and lsTDeHase using fusion PCR

[248] Step 1: Make a DNA construct that contains an *AtGLR1.1* (Locus ID # At3g04110) promoter with a *ssTDeHase* gene and a NOS terminator in the following manner.

[249] Step 1a: Use PCR to amplify the glutamate receptor 1.1 promoter (-1400 to -1 bps) with a short overlap for the 5′ end of ssTDeHase at the 3′ end using 500 ng of genomic DNA isolated from an *Arabidopsis thaliana* Col-0. Add 300 nM of the following primers: 5′AtGLR1.1 (5′-gatcatacatattcatacttgatg-3′; SEQ ID NO:66) and AtGLR1.1ssTDeHase (5′-gagctgtcagtgttttggtcatataatttcttgtatagctctgtaac-3′; SEQ ID NO:67). Run the fusion PCR as described by Szewczyk et al. (45).

[250] Step 1b: Use PCR to amplify the ssTDeHase gene from 500 ng of DNA from *Roseobacter denitrificans*. Add 300 nM of the following primers: 5′ssTDeHase (5′-atgaccaaaacactgacagctc-3′; SEQ ID NO:68) and 3′ssTDeHase (5′-ttaagccttgaagggcgggc-3′; SEQ ID NO:69). Run the fusion PCR as described by Szewczyk et al. (45).

[251] Step 1c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of ssTDeHase at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers ssTDeHaseNOS (5′-gcccgcccttcaaggcttaagctaccgagctcgaatttcc-3′; SEQ ID NO:70) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[252] Step 1d: Combine the PCR fragments (Example 7: 1a, 1b, and 1c) and 300 nM of the following primers MP2 (5′-ttttggtacccgaagctcaatcgtctcgag-3′; SEQ ID NO:71) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[253] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI

[254] Step 2: Make a DNA construct that contains an AtGLR1.1 promoter with a lsTDeHase gene and a NOS terminator to in the following manner.

[255] Step 2a: Use PCR to amplify the *AtGLR1.1* promoter (-1714 to -1 bps) with a short overlap for the 5′ end of lsTDeHase at the 3′ end of the promoter using 500 ng of genomic DNA isolated from an *Arabidopsis thaliana* Col-0. Add 300 nM of the following primers: 5′AtGLR1.1 (5′-gatcatacatattcatacttgatg-3′; SEQ ID NO:66) and AtGLR1.1-lsTDeHase (5′-gtgctttggtctatgtggcatataatttcttgtatagctctgtaac-3′; SEQ ID NO:72). Run the fusion PCR as described by Szewczyk et al. (45).

[256] Step 2b: Use PCR to amplify the *lsTDeHase* gene from 500 ng of DNA from Roseobacter denitrificans. Add 300 nM of the following primers: 5′lsTDeHase (5′-

atgccacatagaccaaagcac-3′; SEQ ID NO:73) and 3′lsTDeHase (5′-tcagagaatttcatcgcgaag-3′; SEQ ID NO:74). Run the fusion PCR as described by Szewczyk et al. (45).

[257] Step 2c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of lsTDeHase at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers lsTDeHaseNOS (5′-cttcgcgatgaaattctctgagctaccgagctcgaatttcc-3′; SEQ ID NO:75) and 3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[258] Step 2d: Combine the PCR fragments (Example 7: 2a, 2b, and 2c) and 300 nM of the following primers NP2 (5′-aaaaatctagacgaagctcaatcgtctcgag-3′; SEQ ID NO:76) and LP5 (5′-aaaaaaagcttgatctagtaacatagatgacac-3′; SEQ ID NO:65). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[259] Step 2e: Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify the plasmid that contains the DNA construct to confirm its identity and the fidelity of the sequence as described in Example 1: step 1e.purify. Digest the plasmid with XbaI and HindIII, isolate the DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XbaI and HindIII.

[260] Step 3: Ligate the *AtGLR1.1* promoter-*ssTDeHase*-NOS terminator construct upstream of the *AtGLR1.1* promoter-*lsTDeHase*-NOS terminator construct into a plant expression vector.

[261] Step 3a. Digest the pCambia2300-*AtGLR1.1* promoter-*ssTDeHase*-NOS terminator clone (from Example 4: Step 1e) with Acc65I and XbaI, isolate DNA insert and ligate it into the vector pCambia2300-AtGLR1.1 promoter-lsTDeHase-NOS terminator (from Example 4: Step 2e) that has been predigested with Acc65I and XbaI. Transform the

DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[262] Step 4: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

Step 5: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 8

Development of a transgenic plant that non-constitutively expresses (AtSULTR1;3 promoter*) TDO using fusion PCR*

[263] Step 1: Make a DNA construct that contains an 5′*AtSULTR1;3* promoter with a *TDO* gene and a NOS terminator in the following manner.

[264] Step 1a: Use PCR to amplify the *AtSULTR1;3* promoter (-2406 to -1 bps) with a short overlap for the 5′ end of TDO at the 3′ end using 500 ng of genomic DNA isolated from an Arabidopsis thaliana Col-0. Add 300 nM of the following primers: 5′AtSULTR1;3 (5′-tcacaatcgatggactctc-3′; SEQ ID NO:77) and 5′AtSULTR1;3 TDO (5′-gtaatgctcagacgttcactcattgctatgtgtgttttgtagc-3′; SEQ ID NO:78). Run the fusion PCR as described by Szewczyk et al. (45).

[265] Step 1b: Use PCR to amplify the *TDO* gene from 500 ng of DNA from E. coli strain K12. Add 300 nM of the following primers: 5′TDO (5′-atgagtgaacgtctgagcattac-3′; SEQ ID NO:79) and 3′TDO (5′-ttaccccgcccgataaaacg-3′; SEQ ID NO:80). Run the fusion PCR as described by Szewczyk et al. (45).

[266] Step 1c: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of ADO at the 5′ end of the terminator using 500 ng of pPV1. Add 300 nM of the following primers TDONOS (5′-cgttttatcgggcggggtaagctaccgagctcgaatttcc-3′; SEQ ID NO:81) and

3′NOS (5′-cacgacgttgtaaaacgacggc-3′; SEQ ID NO:32). Run the fusion PCR as described by Szewczyk et al. (45).

[267] Step 1d: Combine the amplified fragments from Example 3: steps 1a, 1b, and 1c and 300 nM of the following primers OP2 (5′-ttttggtaccctatattggtgtcattttgcc-3′; SEQ ID NO:82) and GP5 (5′-tttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[268] Step 1e. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[269] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[270] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[271] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 9

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO fused in-frame with SAD (without a linker) using fusion PCR

[272] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a *CDO* gene fused in-frame with a SAD gene and a NOS terminator in the following manner.

[273] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of CDO at the 3' end that was amplified in Example 2: Step 1a.

[274] Step 1b: Use the *CDO* gene that was amplified in Example 1: Step 1b.

[275] Step 1c: Use PCR to amplify the SAD gene with a short overlap for the 3' end of CDO at the 5' end using 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: CDO/SAD (5′-gagcgtctcgcaggagaataacatggacgagtctgatgggaagctg -3′; SEQ ID NO:83) and 3′SAD (5′-tcatagatccttcccgagtttc-3′; SEQ ID NO:40). Run the fusion PCR as described by Szewczyk et al. (45).

[276] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of SAD at the 5′ end of the NOS terminator that was amplified in Example 2: Step 2c.

[277] Step 1e: Combine the PCR fragments (Example 9: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and IP5 (5′-ttttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[278] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and SalI, isolate DNA fragment and

ligate into the vector pCAMBIA2300 that has been predigested with XmaI and SalI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[279] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[280] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 10

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO fused in-frame with a linker to SAD using fusion PCR

[281] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a *CDO* gene fused in-frame with a linker and the *SAD* gene and a NOS terminator in the following manner.

[282] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of CDO at the 3' end that was amplified in Example 2: Step 1a.

[283] Step 1b: Use the *CDO* gene that was amplified in Example 1: Step 1b.

[284] Step 1c: Use PCR to amplify the *SAD* gene with a short overlap for the 3' end of CDO at the 5' end using 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: CDOlinkerSAD (5′-gagcgtctcgcaggagaataacagtactgaaggcgaagttaacgcggaagaagaaggctttatggacgagtctgatgggaag ctg -3′; SEQ ID NO:84) and 3′SAD (5′-tcatagatccttcccgagtttc-3′; SEQ ID NO:40). Run the fusion PCR as described by Szewczyk et al. (45).

[285] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of SAD at the 5′ end of the NOS terminator that was amplified in Example 2: Step 2c.

[286] Step 1e: Combine the PCR fragments (Example 10: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and IP5 (5′-ttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[287] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and SalI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[288] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[289] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 11

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) SAD fused in-frame with CDO withlinker using fusion PCR

[290] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a *SAD* gene fused in-frame with a *CDO* gene with a linker and a NOS terminator in the following manner.

[291] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of *SAD* at the 3′ end that was amplified in Example 2: Step 2a.

[292] Step 1b: Step Use the *SAD* gene that was amplified in Example 2: Step 2b.

[293] Step 1c: Use PCR to amplify the CDO gene with a linker and short overlap for the SAD at the 3' using 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: SADlinkerCDO (5′-gaaactcgggaaggatctaagtactgaaggcgaagttaacgcggaagaagaaggctttatggagcagactgaagtcatg-3′; SEQ ID NO:85 and 3′CDO (5′-tcagttattctcctgcgagac-3′; SEQ ID NO:30). Run the fusion PCR as described by Szewczyk et al. (45).

[294] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of CDO at the 5′ end of the NOS terminator that was amplified in Example 1: Step 1c.

[295] Step 1e: Combine the PCR fragments (Example 11: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[296] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and XbaI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[297] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[298] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 12

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO fused in-frame with GAD (without a linker) using fusion PCR

[299] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a *CDO* gene fused in-frame with a *GAD* gene and a NOS terminator in the following manner.

[300] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of CDO at the 3' end that was amplified in Example 2: Step 1a.

[301] Step 1b: Use the *CDO* gene that was amplified in Example 1: Step 1b.

[302] Step 1c: Use PCR to amplify the *GAD* gene with a short overlap for the 3' end of CDO at the 5' end using from 500 ng of DNA from E. coli strain K12. Add 300 nM of the following primers: CDO/GAD (5′-gagcgtctcgcaggagaataacAtggataagaagcaagtaacg-3′; SEQ ID NO:86) and 3′GAD (5′-tcaggtatgtttaaagctgttc-3′; SEQ ID NO:46). Run the fusion PCR as described by Szewczyk et al. (45).

[303] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of GAD at the 5′ end of the NOS terminator that was amplified in Example 3: Step 2c.

[304] Step 1e: Combine the PCR fragments (Example 12: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and IP5 (5′-ttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[305] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and SalI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[306] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[307] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 13

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) CDO fused in-frame with a linker to GAD using fusion PCR

[308] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a CDO gene fused in-frame with a linker and the GAD gene and a NOS terminator in the following manner.

[309] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of CDO at the 3' end that was amplified in Example 2: Step 1a.

[310] Step 1b: Use the *CDO* gene that was amplified in Example 1: Step 1b.

[311] Step 1c: Use PCR to amplify the *GAD* gene with a short overlap for the 3' end of *CDO* at the 5' end using 500 ng of DNA from E. coli strain K12. Add 300 nM of the following primers: CDOlinkerGAD (5′-gagcgtctcgcaggagaataacagtactgaaggcgaagttaacgcggaagaagaaggctttatggataagaagcaagtaacg

-3′; SEQ ID NO:87) and 3′GAD (5′-tcaggtatgtttaaagctgttc-3′; SEQ ID NO:46). Run the fusion PCR as described by Szewczyk et al. (45).

[312] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of GAD at the 5′ end of the NOS terminator that was amplified in Example 3: Step 2c.

[313] Step 1e: Combine the PCR fragments (Example 13: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and IP5 (5′-ttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[314] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and SalI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[315] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[316] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 14

Development of a transgenic plant that constitutively expresses (AtPHYB promoter) GAD fused in-frame with a linker to CDO using fusion PCR

[317] Step 1: Make a DNA construct that contains an *AtPHYB* (Locus ID# At2g18790) promoter with a GAD gene fused in-frame with a linker and the CDO gene and a NOS terminator in the following manner.

[318] Step 1a: Use the *AtPHYB* promoter (–1960 to -1 bps) with a short overlap for the 5' end of GAD at the 3' end that was amplified in Example 3: Step 2a.

[319] Step 1b: Step Use the *GAD* gene that was amplified in Example 3: Step 2b.

[320] Step 1c: Use PCR to amplify the *GAD* gene with a linker and short overlap for the *CDO* at the 3' using 500 ng of cDNA from a zebrafish (*Danio rerio*) cDNA library. Add 300 nM of the following primers: GADlinkerCDO (5′-gaacagctttaaacataccagtactgaaggcgaagttaacgcggaagaagaaggctttatggagcagactgaagtcatg -3′; SEQ ID NO:88) and 3′GAD (5′-tcaggtatgtttaaagctgttc-3′; SEQ ID NO:46). . Run the fusion PCR as described by Szewczyk et al. (45).

[321] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of CDO at the 5′ end of the NOS terminator that was amplified in Example 1: Step 1c.

[322] Step 1e: Combine the PCR fragments (Example 14: 1a, 1b, 1c, and 1d) and 300 nM of the following primers HP2 (5′-ttttcccgggattcttgaattacgattgtacc-3′; SEQ ID NO:37) and IP5 (5′-ttttttgtcgacgatctagtaacatagatgacac-3′; SEQ ID NO:43). Run the fusion PCR as described by Szewczyk et al. (45). Clone into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[323] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with XmaI and SalI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with XmaI and SalI. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[324] Step 2: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[325] Step 3: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 15

Development of a transgenic plant that non-constitutively expresses (AtGAD2 promoter) SA fused in-frame to TPAT using fusion PCR

[326] Step 1: Make a DNA construct that contains an *AtGAD2* (Locus ID# At1g65960) promoter with a *SA* gene fused in-frame with a *TPAT* gene and a NOS terminator in the following manner.

[327] Step 1a: Use the *AtGAD2* promoter (-1714 to -1 bps) with a short overlap for the 5′ end of SA at the 3′ end that was amplified in Example 6: Step 2a.

[328] Step 1b: Step Use the *SA* gene that was amplified in Example 6: Step 2b.

[329] Step 1c: Use PCR to amplify the *TPAT* gene with a short overlap for the 3′end of *SA* at the 5′end using 500 ng of DNA from *Roseobacter denitrificans* strain. Add 300 nM of the following primers: SA/TPAT (5′-catgcgtccacagactgtcatggacggcaatttcaatg-3′; SEQ ID NO:89) and 3′TPAT (5′-ttagccgaaaacgcgcgacag-3′; SEQ ID NO:57). Run the fusion PCR as described by Szewczyk et al. (45).

[330] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of *TPAT* at the 5′ end of the NOS terminator that was amplified in Example 5: Step 1c.

[331] Step 1e: Combine the amplified fragments (Example 15: steps 1a, 1b, 1c and 1d) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR

as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[332] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[333] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[334] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[335] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 16

Development of a transgenic plant that non-constitutively expresses (AtGAD2 promoter) SA fused in-frame with a linker to TPAT using fusion PCR

[336] Step 1: Make a DNA construct that contains an *AtGAD2* (Locus ID# At1g65960) promoter with a SA gene fused in-frame with a linker to a *TPAT* gene and a NOS terminator in the following manner.

[337] Step 1a: Use the *AtGAD2* promoter (-1714 to -1 bps) with a short overlap for the 5′ end of SA at the 3′ end that was amplified in Example 6: Step 2a.

[338] Step 1b: Use the *SA* gene that was amplified in Example 6: Step 2b.

[339] Step 1c: Use PCR to amplify the *TPAT* gene with a linker and short overlap for the 3′end of SA at the 5′end using 500 ng of DNA from *Roseobacter denitrificans* strain. Add 300 nM of the following primers: SAlinkerTPAT (5′-catgcgtccacagactgtcagtactgaaggcgaagttaacgcggaagaagaaggctttatggacggcaatttcaatg-3′; SEQ ID NO:90) and 3′TPAT (5′-ttagccgaaaacgcgcgacag-3′; SEQ ID NO:57). Run the fusion PCR as described by Szewczyk et al. (45).

[340] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of *TPAT* at the 5′ end of the NOS terminator that was amplified in Example 5: Step 1c.

[341] Step 1e: Combine the amplified fragments (Example 16: steps 1a, 1b, 1c and 1d) 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[342] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[343] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[344] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[345] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 17

Development of a transgenic plant that non-constitutively expresses (AtGAD2 promoter) TPAT fused in-frame with a linker to a SA gene using fusion PCR

[346] Step 1: Make a DNA construct that contains an *AtGAD2* (Locus ID# At1g65960) promoter with a *TPAT* gene fused in-frame with a linker to a SA gene and a NOS terminator in the following manner.

[347] Step 1a: Use the *AtGAD2* promoter (-1714 to -1 bps) with a short overlap for the 5′ end of TPAT at the 3′ end that was amplified in Example 5: Step 1a.

[348] Step 1b: Use the *TPAT* gene that was amplified in Example 5: Step 1b.

[349] Step 1c: Use PCR to amplify the SA with a short overlap for the 3′ end of TPAT with a linker at the 5' end using 500 ng of DNA from *Roseobacter denitrificans* strain. Add 300 nM of the following primers: TPATlinkerSA (5′-cgctgtcgcgcgttttcggcagtactgaaggcgaagttaacgcggaagaagaaggctttatgaaaatgaccactgaag-3′; SEQ ID NO:91) and 3′SA (5′-tcagacagtctgtggacgc-3′; SEQ ID NO:62). Run the fusion PCR as described by Szewczyk et al. (45).

[350] Step 1d: Use PCR to amplify the NOS terminator with a short overlap for the 3′ end of SA that was amplified in Example 6: Step2c.

[351] Step 1e: Combine the amplified fragments (Example 17: steps 1a, 1b, 1c and 1d) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[352] Step 1f. Transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment

and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[353] Step 2. Transform the DNA construct into *E. coli*, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[354] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[355] Step 4: Transform plant, *Arabidopsis thaliana:* Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 18

Development of a transgenic plant that non-constitutively expresses (AtGLR1.1 promoter) ssTDeHase fused in-frame with lsTDeHase (without a linker) using fusion PCR

[356] Step 1: Make a DNA construct that contains an *AtGLR1.1* (Locus ID # At3g04110) promoter with a *ssTDeHase* gene fused in-frame with a *lsTDeHase* gene and a NOS terminator in the following manner.

[357] Step 1a: Use the *AtGLR1.1* promoter (–1960 to -1 bps) with a short overlap for the 5' end of *ssTDeHase* at the 3' end that was amplified in Example 7: Step 1a.

[358] Step 1b: Use the *ssTDeHase* gene that was amplified in Example 7: Step 1b.

[359] Step 1c: Use PCR to amplify the *lsTDeHase* gene with a short overlap for the 3' end of *ssTDeHase* at the 5' end using of DNA from *Roseobacter denitrificans*. Add 300 nM of the following primers: ssTDeHase/ lsTDeHase (5′-gcccgcccttcaaggctatgccacatagaccaaagcac-3′; SEQ ID NO:92) and 3′lsTDeHase (5′-

tcagagaatttcatcgcgaag-3′; SEQ ID NO:74). Run the fusion PCR as described by Szewczyk et al. (45).

[360] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of lsTDeHase at the 5′ end of the NOS terminator that was amplified in Example 7: Step 2c.

[361] Step 1e: Combine the amplified fragments (Example 18: steps 1a, 1b, 1c and 1d) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-tttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[362] Step 1f. Transform E. coli, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[363] Step 2. Transform the DNA construct into E. coli, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[364] Step 3: Transform Agrobacterium tumefaciens: Transform the DNA construct into Agrobacterium tumefaciens, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[365] Step 4: Transform plant, Arabidopsis thaliana: Transform the construct into Arabidopsis thaliana, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 19

Development of a transgenic plant that non-constitutively expresses (AtGLR1.1 promoter) ssTDeHase fused in-frame with a linker to lsTDeHase using fusion PCR

[366] Step 1: Make a DNA construct that contains an *AtGLR1.1* (Locus ID # At3g04110) promoter with a *ssTDeHase* gene fused in-frame with a linker and the *lsTDeHase* gene and a NOS terminator in the following manner.

[367] Step 1a: Use the *AtGLR1.1* promoter (–1960 to -1 bps) with a short overlap for the 5' end of *ssTDeHase* at the 3' end that was amplified in Example 7: Step 1a.

[368] Step 1b: Use the *ssTDeHase* gene that was amplified in Example 7: Step 1b.

[369] Step 1c: Use PCR to amplify the *lsTDeHase* gene with a short overlap for the 3' end of *ssTDeHase* at the 5' end using of DNA from *Roseobacter denitrificans*. Add 300 nM of the following primers: ssTDeHaselinkerlsTDeHase (5′-gcccgcccttcaaggctagtactgaaggcgaagttaacgcggaagaagaaggctttatgccacatagaccaaagcac -3′; SEQ ID NO:93) and 3′lsTDeHase (5′-tcagagaatttcatcgcgaag-3′; SEQ ID NO:74). Run the fusion PCR as described by Szewczyk et al. (45).

[370] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of lsTDeHase at the 5′ end of the NOS terminator that was amplified in Example 7: Step 2c.

[371] Step 1e: Combine the amplified fragments (Example 19: steps 1a, 1b, 1c and 1d) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-ttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[372] Step 1f. Transform E. coli, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment

and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[373] Step 2. Transform the DNA construct into E. coli, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[374] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into Agrobacterium tumefaciens, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[375] Step 4: Transform plant, *Arabidopsis thaliana*: Transform the construct into *Arabidopsis thaliana*, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

EXAMPLE 20

Development of a transgenic plant that non-constitutively expresses (*AtGLR1.1* promoter) lsTDeHase fused in-frame with a linker to ssTDeHase using fusion PCR

[376] Step 1: Make a DNA construct that contains an *AtGLR1.1* (Locus ID # At3g04110) promoter with a lsTDeHase gene fused in-frame with a linker and the ssTDeHase gene and a NOS terminator in the following manner.

[377] Step 1a: Use the *AtGLR1.1* promoter (–1960 to -1 bps) with a short overlap for the 5' end of *lsTDeHase* at the 3' end that was amplified in Example 7: Step 2a.

[378] Step 1b: Use the *lsTDeHase* gene that was amplified in Example 7: Step 2b.

[379] Step 1c: Use PCR to amplify the *lsTDeHase* gene with a short overlap for the 3' end of *ssTDeHase* at the 5' end using of DNA from *Roseobacter denitrificans*. Add 300 nM of the following primers: lsTDeHaselinkerssTDeHase (5′-cttcgcgatgaaattctcagtactgaaggcgaagttaacgcggaagaagaaggctttatgaccaaaacactgacagctc-3′; SEQ ID NO:94) and 3′ssTDeHase (5′-ttaagccttgaagggcgggc-3′; SEQ ID NO:69). Run the fusion PCR as described by Szewczyk et al. (45).

[380] Step 1d: Use the NOS terminator with a short overlap for the 3′ end of ssTDeHase at the 5′ end of the NOS terminator that was amplified in Example 7: Step 1c.

[381] Step 1e: Combine the amplified fragments (Example 3: steps 1a, 1b, 1c and 1d) and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-tttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[382] Step 1f: Combine the amplified fragments from Example 20: steps 1a, 1b, 1c and 1d and 300 nM of the following primers KP2 (5′-ttttggtaccctctttcggaacgagcttcaac-3′; SEQ ID NO:59) and GP5 (5′-tttttttttctagagatctagtaacatagatgacac-3′; SEQ ID NO:34). Run the fusion PCR as described by Szewczyk et al. (45). Clone the amplified DNA fragment into pCR4.0-TOPO as described by the manufacturer (Invitrogen).

[383] Step 1g. Transform E. coli, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, purify DNA and sequence (described in Example 1: step 1e). Digest the plasmid with Acc65I and XbaI, isolate DNA fragment and ligate into the vector pCAMBIA2300 that has been predigested with Acc65I and XbaI.

[384] Step 2. Transform the DNA construct into E. coli, select for antibiotic resistance and confirm the presence of the DNA construct with PCR or by restriction digest analysis.

[385] Step 3: Transform *Agrobacterium tumefaciens*: Transform the DNA construct into Agrobacterium tumefaciens, select for antibiotic resistance and confirm the presence of the DNA construct as described in Example 1: Step 2.

[386] Step 4: Transform plant, *Arabidopsis thaliana*: Transform the construct into Arabidopsis thaliana, select for antibiotic resistance, select for homozygote plants and confirm the presence of the DNA constructs as described in Example 1: Step 3.

CLAIMS

1. An isolated cell comprising a first exogenous DNA which comprises two separate expression cassettes, wherein a first expression cassette comprises a first promoter operably linked to a first polynucleotide and a second expression cassette comprises a second promoter operably linked to a second polynucleotide, wherein:

(i) the first polynucleotide encodes cysteine dioxygenase (CDO); and the second polynucleotide encodes sulfinoalanine decarboxylase (SAD); or

(ii) the first polynucleotide encodes cysteine dioxygenase (CDO); and the second polynucleotide encodes glutamate decarboxylase (GAD); or

(iii) the first polynucleotide encodes taurine-pyruvate aminotransferase (TPAT) and the second polynucleotide encodes sulfoacetaldehyde acetyltransferase (SA); or

(iv) the first polynucleotide encodes a small subunit of taurine dehydrogenase (ssTDeHase) and the second polynucleotide encodes a large subunit of taurine dehydrogenase (lsTDeHase).

2. An isolated cell comprising a first exogenous DNA which comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes (i) CDO and SAD; (ii) CDO and GAD; (iii) TPAT; (iv) TPAT and SA; (v) ssTDeHase and lsTDeHase or (vi) fused CDO–SAD; (vii) fused CDO-linker-SAD; (viii) fused SAD-CDO; (ix) fused SAD-linker-CDO; (x) fused CDO–GAD; (xi) fused CDO-linker-GAD; (xii) fused GAD-CDO; (xiii) fused GAD-linker-CDO; (xiv) fused TPAT–SA; (xv) fused TPAT-linker-SA; (xvi) fused SA-TPAT; (xvii) fused SA-linker-TPAT;

(xviii) fused ssTDeHase–lsTDeHase; (ixx) fused ssTDeHase-linker-lsTDeHase; (xx) fused lsTDeHase-ssTDeHase; or (xxi) fused lsTDeHase-linker-ssTDeHase:

3. An isolated cell comprising a first exogenous DNA which comprises a promoter operably linked to a polynucleotide, wherein the polynucleotide encodes CDO, ADO, or taurine dioxygenase (TDO) and wherein the isolated cell is a plant cell.

4. The isolated cell of claim 1 or claim 2 wherein:

the CDO comprises the amino acid sequence SEQ ID NO:3 or the amino acid sequence SEQ ID NO:4;

the SAD comprises the amino acid sequence SEQ ID NO:7 or the amino acid sequence SEQ ID NO:8;

the GAD comprises the amino acid sequence SEQ ID NO:11 or the amino acid sequence SEQ ID NO:12;

the TPAT comprises the amino acid sequence SEQ ID NO:18;

the SA comprises the amino acid sequence SEQ ID NO:20;

the ssTdeHase comprises the amino acid sequence SEQ ID NO:22; and/or

the lsTdeHase comprises the amino acid sequence SEQ ID NO:24.

5. The isolated cell of claim 3 wherein:

the CDO comprises the amino acid sequence SEQ ID NO:3 or the amino acid sequence SEQ ID NO:4;

the ADO comprises the amino acid sequence SEQ ID NO:13 or the amino acid sequence SEQ ID NO:14; and/or

the TDO comprises the amino acid sequence SEQ ID NO:25.

6. The isolated cell of claim 1 wherein the first polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:17, and SEQ ID NO:21.

7. The isolated cell of claim 1 wherein the second polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23.

8. The isolated cell of claim 2 wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23.

9. The isolated cell of claim 3 wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:25.

10. The isolated cell of claim 1 wherein at least one of the first and second promoters is a constitutive promoter.

11. The isolated cell of claim 2 or claim 3 wherein the promoter is a constitutive promoter.

12. The isolated cell of claim 1 wherein at least one of the first and second promoters is a non-constitutive promoter.

13. The isolated cell of claim 2 or claim 3 wherein the promoter is a non-constitutive promoter.

14. The isolated cell of claim 12 or claim 13 wherein the non-constitutive promoter is selected from the group consisting of a tissue-preferred promoter, a tissue-specific promoter, a cell type-specific promoter, an inducible promoter, a plant glutamate decarboxylase (GAD) promoter, plant sulphate transporter (SULTR) promoter, and a plant glutamate receptor (GLR) promoter.

15. The isolated cell of claim 1 which is a plant cell.

16. The isolated cell of claim 1 which is a eukaryotic cell.

17. The isolated cell of claim 1 which is a prokaryotic cell.

18. The isolated cell of claim 1 wherein one or both of the first and second polynucleotides comprises a subcellular location sequence.

19. The isolated cell of claim 2 or claim 3 wherein the polynucleotide comprises a subcellular location sequence.

20. The isolated cell of claim 18 or claim 19 wherein the subcellular location sequence targets a subcellular location selected from the group consisting of an apoplast, a vacuole, a plastid, a chloroplast, a proplastid, an etioplast, a chromoplast, a mitochondrion, a peroxisome, a glyoxysome, a nucleus, a lysosome, an endomembrane system, an endoplasmic reticulum, a vesicle, and a Golgi apparatus.

21. A plant storage organ comprising the isolated cell of claim 1, claim 2, or claim 3.

22. The plant storage organ of claim 21 which a seed, a tuber, a fruit, or a root.

23. A transgenic seed having in its genome exogenous DNA encoding a protein selected from the group consisting of CDO, SAD, GAD, ADO, TPAT, SA, TDO, and TDeHase, wherein

enzymatic activity of the protein increases production of taurine or hypotaurine in a transgenic plant grown from the transgenic seed.

24. The transgenic seed of claim 23 wherein:

the CDO comprises the amino acid sequence SEQ ID NO:3 or SEQ ID NO:4;

the SAD comprises the amino acid sequence SEQ ID NO:7 or SEQ ID NO:8;

the GAD comprises the amino acid sequence SEQ ID NO:11 or SEQ ID NO:12;

the ADO comprises the amino acid sequence SEQ ID NO:15 or SEQ ID NO:16;

the TPAT comprises the amino acid sequence SEQ ID NO:18;

the SA comprises the amino acid sequence SEQ ID NO:20;

the TDO comprises the amino acid sequence SEQ ID NO:26; and/or

the TDeHase comprises the amino acid sequence SEQ ID NO:22 or SEQ ID NO:24.

25. The transgenic seed of claim 23 wherein the exogenous DNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25.

26. A transgenic plant grown from the transgenic seed of any of claim 23.

27. The transgenic plant of claim 26 which is selected from the group consisting of acacia, alfalfa, algae, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beech, beet, Bermuda grass, blackberry, blueberry, Blue grass, broccoli, brussels sprouts, cabbage, camelina, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, duckweed, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, fescue, figs, forest trees, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, maize, mango, melon, mushroom, nectarine, nut, oat, okra, onion, orange, ornamental plants, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, rye grass, seaweed, scallion, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, switchgrass, tangerine, tea, tobacco, tomato, turf, turnip, a vine, watermelon, wheat, yam, and zucchini.

28. A method of producing a crop of transgenic plants, comprising multiplying or breeding plant material to obtain a crop of transgenic plants from the transgenic seed of claim 23.

29. A method of producing taurine or hypotaurine, comprising growing the isolated cell of claim 1 under conditions which permit expression of the first and second polynucleotides, thereby producing taurine or hypotaurine.

30. A method of producing taurine or hypotaurine, comprising growing the isolated cell of claim 2 or claim 3 under conditions which permit expression of the polynucleotide, thereby producing taurine or hypotaurine.

31. The method of claim 29 or claim 30 further comprising introducing the exogenous DNA into the isolated cell.

32. A transgenic plant having in its genome exogenous DNA encoding a protein selected from the group consisting of CDO, SAD, GAD, ADO, TPAT, SA, TDO, and TDeHase wherein the plant has increased growth, yield, or biomass, altered development, or increased tolerance to biotic (pests, pathogens, bacteria, microbes, viruses, viroids, microorganisms, invertebrates, insects, nematodes, or vertebrates) or abiotic (changes in osmotic conditions, oxidative damage, drought, salt, cold, freezing, heat, UV light or light intensity) stress.

33. A transgenic plant having in its genome exogenous DNA encoding a protein selected from the group consisting of CDO, SAD, GAD, ADO, TPAT, SA, TDO, and TDeHase wherein the plant has increased water-use-efficiency or nitrogen-use-efficiency.

34. A transgenic plant having in its genome exogenous DNA encoding a protein selected from the group consisting of CDO, SAD, GAD, ADO, PAT, SA, TDO, and TDeHase wherein the seeds have increased oil, starch or protein.

35. A method of altering a property of a transgenic plant, comprising contacting a transgenic plant having in its genome exogenous DNA encoding a protein selected from the group consisting of CDO, SAD, GAD, ADO, TPAT, SA, TDO, and TDeHase with an agent which increases sulfur or nitrogen concentration in cells of the transgenic plant, wherein the property is selected from the group consisting of increased plant growth, altered plant development, increased tolerance to biotic stress, increased tolerance to abiotic stress, increased yield, and increased biomass.

36. The method of claim 35 wherein the agent increases sulfur concentration and the agent is selected from the group consisting of fertilizer, sulfur, sulfite, sulfide, sulfate, taurine, hypotaurine, homotaurine, homocysteine, cystathionine cysteate, 2-sulfacetaldehyde, N-acetyl thiazolidine 4 carboxylic acid (ATCA), glutathione, and bile.

37. The method of claim 35 wherein the agent increases nitrogen concentration and the agent is selected from the group consisting of an amino acid and a polyamine.

38. The method of claim 37 wherein the agent is an amino acid and the amino acid is not naturally present in a protein.

39. The method of claim 38 wherein the amino acid is GABA, citrulline, or ornithine.

40. The method of claim 37 wherein the agent is an amino acid and the amino acid is selected from the group consisting of cysteine, methionine, glutamate, glutamine, serine, alanine, and glycine.

41. A pharmaceutical composition comprising an extract of the transgenic plant of claim 26, wherein the extract comprises taurine, hypotaurine, homotaurine, taurine derivatives, taurine conjugates, or taurine polymers.

42. A nutritional supplement comprising an extract of the transgenic plant of claim 26.

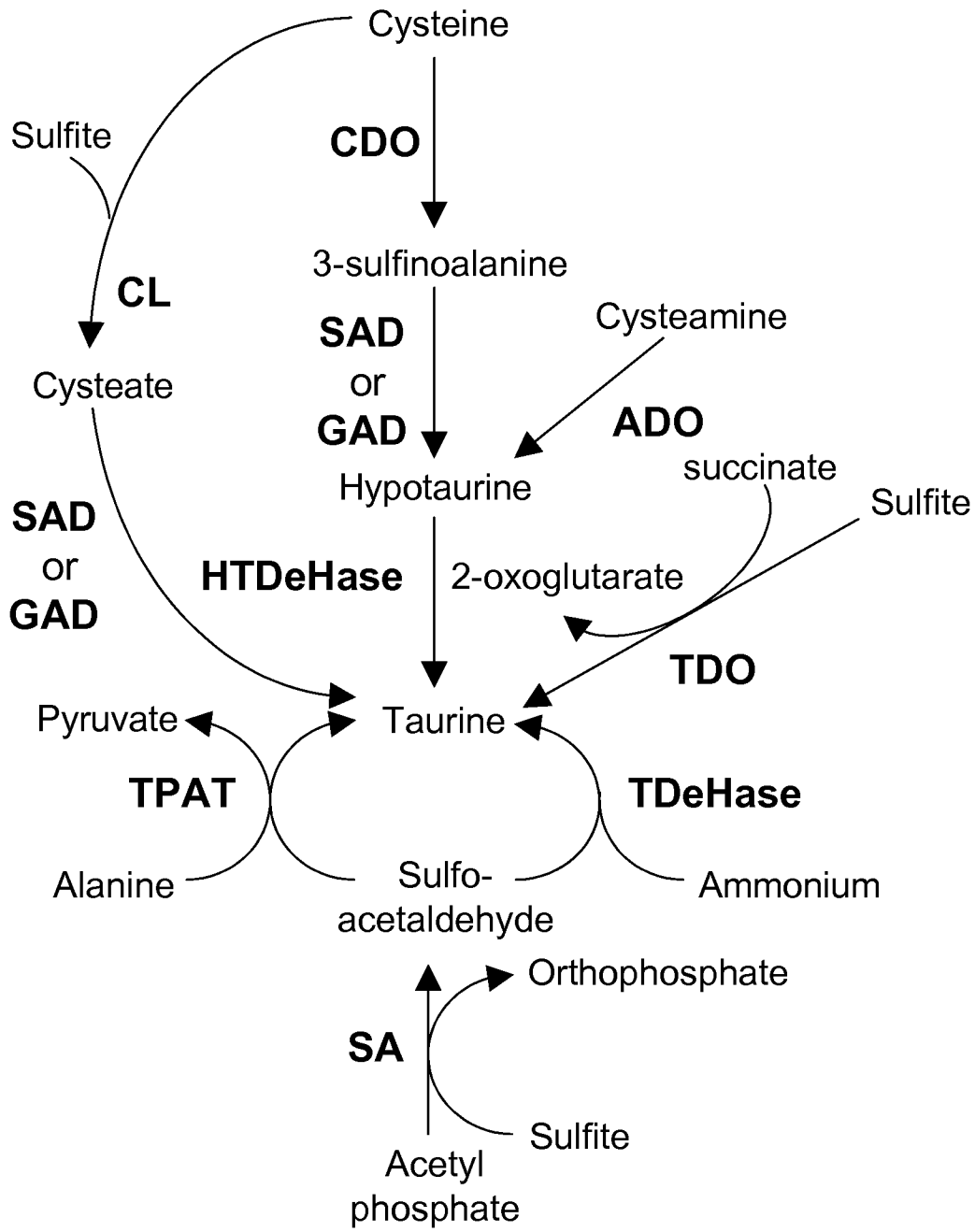
43. An animal feed supplement comprising a component selected from the group consisting of :

the plant storage organ of claim 21;

the transgenic seed of claim 23; and

the transgenic plant of claim 26.

FIGURE 1


Cysteine
Sulfite
CDO
3-sulfinoalanine
CL
SAD
or
GAD
Cysteamine
Cysteate
ADO
Hypotaurine
succinate
Sulfite
SAD
or
GAD
HTDeHase
2-oxoglutarate
TDO
Pyruvate
Taurine
TPAT
TDeHase
Alanine
Sulfo-
acetaldehyde
Ammonium
Orthophosphate
SA
Sulfite
Acetyl
phosphate