(12) United States Patent

Ogawa
(10) Patent No.: US 8,268,748 B2
(45) Date of Patent:

Sep. 18, 2012
(54) COMPOSITION FOR PRODUCTION OF PLANT BODY HAVING IMPROVED SUGAR CONTENT, AND USE THEREOF
(75) Inventor: Kenichi Ogawa, Kyoto (JP)
(73) Assignees: Japan Science and Technology Agency, Kawaguchi (JP); Okayama Prefecture, Okayama (JP)
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 134 days.
(21) Appl. No.: $\quad \mathbf{1 2 / 5 9 9}, \mathbf{7 1 0}$
(22) PCT Filed: Nov. 7, 2008
(86) PCT No.: PCT/JP2008/070312
§ 371 (c)(1),
(2), (4) Date: $\quad$ Nov. 11, 2009
(87) PCT Pub. No.: WO2009/063806

PCT Pub. Date: May 22, 2009
Prior Publication Data
US 2010/0242141 A1 Sep. 23, 2010
(30) Foreign Application Priority Data

Nov. 13, 2007
(JP) $\qquad$ 2007-294797
(51) Int. Cl. A01H 3/04
(2006.01)
(52) U.S. Cl.

504/116.1
(58) Field of Classification Search $\qquad$ None See application file for complete search history.

## References Cited

U.S. PATENT DOCUMENTS

| 5,350,689 | A | 9/1994 | Shillito et al. |
| :---: | :---: | :---: | :---: |
| 5,595,733 | A | 1/1997 | Carswell et al. |
| 5,766,900 | A | 6/1998 | Shillito et al. |
| 5,770,450 | A | 6/1998 | Shillito et al. |
| 5,824,302 | A | 10/1998 | Carswell et al. |
| 7,479,267 | B2* | 1/2009 | Ogawa et al. |
| 3/0110527 | Al | 6/2003 | Ogawa et al. |

2004/0052774 A1 3/2004 Creissen et al.
2009/0099023 A1 4/2009 Ogawa et al.
2010/0016166 A1* 1/2010 Ogawa et al.
504/320

| FOREIGN PATENT DOCUM |  |
| :---: | :---: |
| 0655196 A2 | $5 / 1995$ |
| $10-27194$ |  |
| $10-271924$ |  |
| $10 / 1998$ |  |
| $2004-352679$ |  |
| 2126047 | 1098 |
| Cl | $2 / 2004$ |
| WO 2004/016726 |  |
|  | $2 / 2004$ |

## OTHER PUBLICATIONS

Simoni et al (J. Biol. Chem, vol. 277, No. 24, 2002.*
Wingle et al (Planta 1996, 198 151-157).*
Hopkins et al-J. Biol. Chem.(1922) 54, 527-563.*
Examiner's Report for corresponding Australian Application No. 2008321944 dated Sep. 13, 2010.
Ito, H. et al. "The Sugar-Metabolic Enzymes Aldolase and TriosePhosphate Isomerase are Targets of Glutathionylation in Arabidopsis thaliana; Detection using Biotinylated Glutathione", Plant Cell Physiol. (2003) 44(7); p. 655-660 (2003).
Ogawa, K. et al. "Fructose-1,6-Bisphosphate Aldolase is a Target Protein of Glutathionylation in Arabidopsis Chloroplasts", XP003016751, $13^{\text {th }}$ International Congress on Photosynthesis, HTTP://abstracts.co.allenpress.com/pweb/pwc2004/document/ ?ID+39705 (2007).
Supplementary European Search Report for corresponding European Application No. 08849628, issued Dec. 15, 2011.
The Notice of Allowance dated Oct. 4, 2011, for corresponding Russian Patent Application No. 2009139630 , and English Translation.

* cited by examiner

Primary Examiner - Anne Kubelik
Assistant Examiner - Lee A Visone
(74) Attorney, Agent, or Firm - Edwards Wildman Palmer LLP; David G. Conlin; Lisa Swiszcz

## (57)

## ABSTRACT

The composition, in accordance with the present invention, for producing a plant body having an improved sugar content includes glutathione, a polynucleotide encoding $\gamma$-glutamylcysteine synthetase, or a polynucleotide encoding glu-tathione-binding plastid type fructose-1,6-bisphosphate aldolase. The composition preferably includes oxidized glutathione. This allows provision of a composition for easily producing a plant body having an improved sugar content.

12 Claims, 7 Drawing Sheets

FIG. 1


FIG. 2


FIG. 3


FIG. 4


FIG. 5


FIG. 6


FIG. 7


FIG. 8


FIG. 9


## COMPOSITION FOR PRODUCTION OF PLANT BODY HAVING IMPROVED SUGAR CONTENT, AND USE THEREOF

## TECHNICAL FIELD

The present invention relates to a composition, including a substance for regulating an oxidation-reduction state of a cell, which is for producing a plant body having an improved sugar content. The present invention also relates to use of the composition.

## BACKGROUND ART

A plant such as fruit, vegetable, and cereal generally includes sugar. An amount of sugar in the plant is represented by a sugar content. The sugar content affects a commercial value of plant depending on a type of the plant. Therefore, in recent years, technical developments for increasing a sugar content of a plant have been carried out.

For example, tomatoes of high sugar content are produced mainly by soil culture. Further, a technique for producing tomatoes of high sugar content by nutrient solution culture has been suggested (Patent Literature 1).

It is known that a substance for regulating an oxidationreduction state of a cell, such as glutathione, can function as a differentiation control agent for a cell or an organ (Patent Literature 2). Further, it is known that glutathione can function as a plant growth control auxiliary agent (Patent Literature 3 ).

## CITATION LIST

Patent Literature 1
Japanese Patent Application Publication, Tokukaihei, No. 10-271924 (Publication Date: Oct. 13, 1998)

Patent Literature 2
International Publication WO 01/080638 (Publication Date: Nov. 1, 2001)

## Patent Literature 3

Japanese Patent Application Publication, Tokukai No. 2004352679 (Publication Date: Dec. 16, 2004)

## SUMMARY OF INVENTION

However, the conventional technique for improving a sugar content of a plant lacks in simplicity. Those who can produce tomatoes of high sugar content by soil culture are limited to few specialists. Further, production of tomatoes of high sugar content by nutrient solution culture requires a specialized technique and specialized production apparatus for cultivation management.

The present invention has been accomplished in view of such circumstances, and an object of the present invention is to provide a composition for easily producing a plant having an improved sugar content and to provide a technique using the composition.

In order to attain the object, the inventors of the present invention studied diligently. As a result, they found that a sugar content of a plant body was improved in a case where the plant body was grown in a culture medium (which includes soil and a soil improvement agent) to which a sub-
stance for regulating an oxidation-reduction state of a cell is added, or in a case where the plant body was sprayed or directly coated with the substance. The present invention was accomplished based on this totally new finding and includes the following inventions.

The composition in accordance with the present invention is a composition for producing a plant body having an improved sugar content, the composition including a substance (excluding hydrogen peroxide) for regulating an oxi-dation-reduction state of a cell.

The composition in accordance with the present invention is preferably arranged so that the substance is glutathione, a polynucleotide encoding $\gamma$-glutamylcysteine synthetase, or a polynucleotide encoding glutathione-binding plastid type fructose-1,6-bisphosphate aldolase

The composition in accordance with the present invention is preferably arranged so that the substance is oxidized glutathione.

The kit in accordance with the present invention is a kit for producing a plant body having an improved sugar content, the kit including a substance (excluding hydrogen peroxide) for regulating an oxidation-reduction state of a cell.

The production method in accordance with the present invention is a method for producing a plant body having an improved sugar content, the method including the step of cultivating the plant body by using a substance (excluding hydrogen peroxide) for regulating an oxidation-reduction state of a cell.

The present invention also includes a plant body obtained by the production method in accordance with the present invention.

Additional objects, features, and strengths of the present invention will be made clear by the description below. Further, the advantages of the present invention will be evident from the following explanation in reference to the drawings.

## BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 illustrates a determination result of sugar content of Lycopersicum esculentum fruit obtained in Example 2.

FIG. 2 illustrates a result of ANOVA analysis on the determination result of sugar content shown in FIG. 1.

FIG. $\mathbf{3}$ is a view illustrating a determination result of relation between sugar content and the number of days from a treatment day of GSSG or GSH.

FIG. 4 illustrates a determination result of starch and glucose of 35 S-GSH1.
FIG. 5 illustrates a determination result of sugar content of Prunus avium fruit obtained in Example 8.
FIG. 6 illustrates a determination result of sugar content of Citrus unshiu fruit obtained in Example 9.

FIG. 7 illustrates a determination result of sugar content of Fragaria ananassa fruit obtained in Example 10.

FIG. 8 illustrates a determination result of sugar content of Zea mays L. var. saccharata Sturt fruit obtained in Example 11.

FIG. 9 is a view illustrating a genetic family tree of the genes of SEQ ID NO: 15 through 36.

## DESCRIPTION OF EMBODIMENTS

1. Composition, in Accordance with the Present

Invention, for Producing Plant Body Having Improved Sugar Content

A composition, in accordance with the present invention, for producing a plant body having an improved sugar content
(hereinafter referred to as "composition in accordance with the present invention") only has to include a substance for regulating an oxidation-reduction state of a cell.

By using the composition in accordance with the present invention, it becomes possible to easily produce a plant body having an improved sugar content. For example, the plant body can be produced in a culture medium that includes the composition in accordance with the present invention. Further, in a case where the substance for regulating an oxida-tion-reduction state of a cell is a polynucleotide as described later, what is necessary to do is only to introduce the polynucleotide into a plant by means of a conventional transformation technique and then grow the plant. This makes it possible to obtain the plant having an improved sugar content in an extremely simple way compared to the conventional technique such as the soil culture described above. This is because this case does not require skills, specialized techniques, specialized production apparatuses, or the like.

In the present invention, the substance for regulating an oxidation-reduction state of a cell is used for the purpose of production of a plant having an improved sugar content. This usage of the substance is new and totally differs from a conventional usage of the substance. Such an effect that the plant having an improved sugar content can be obtained could not have been expected from the conventional usage. Therefore, the present invention is accomplished based on a totally new finding by the inventors of the present invention.

In the present specification, the "plant body having an improved sugar content" is a plant body having a better sugar content than a wild strain of the plant body. In other words, the "plant body having an improved sugar content" has a higher sugar content than the wild strain. That is to say, the composition in accordance with the present invention is a composition used in production of a plant body having a higher sugar content than a wild strain. For example, by cultivating a plant body by using the composition in accordance with the present invention, it is possible to improve a sugar content of the plant body compared to a case of cultivating the plant body without the composition in accordance with the present invention. It is possible to determine a sugar content by a conventional method. It is also possible to determine a sugar content by using a conventional brix refractometer as described in Examples.

In the present specification, the "substance for regulating an oxidation-reduction state of a cell" is a substance that regulates oxidation/reduction of a substance that is responsible for oxidation-reduction of the cell. The substance for regulating an oxidation-reduction state of a cell includes substances that change values of, for example, an occurrence frequency of active oxygen, an absolute amount of glutathione, a ratio between reduced glutathione and oxidized glutathione, an absolute amount of reduced nicotinamide adenine dinucleotide phosphate $(\mathrm{NAD}(\mathrm{P}) \mathrm{H})$, a ratio of NADPH/NADP + , a ratio of oxidized cytochrome c to reduced cytochrome c, and a ratio between oxidation and reduction of a component of electron transfer chain such as plastoquinone and ubiquinone. The substance responsible for oxidation-reduction of a cell is known in the art, but is not limited to those known in the art. The substances that change the values may be, for example, a substance that affects synthesis of glutathione or an amount of glutathione, a substance that promotes or inhibits synthesis of active oxygen, and a substance that promotes or inhibits change of a certain compound into either an oxidized form or a reduced form.

The substance, included in the composition in accordance with the present invention, for regulating an oxidation-reduction state of a cell is not limited as long as being included in
the above-mentioned meaning. However, it is preferable that the substance affects synthesis of glutathione or an amount of glutathione. Such a substance makes it possible to obtain a plant having a higher sugar content.

In the present specification, the "substance that affects synthesis of glutathione or an amount of glutathione" is a substance that changes an amount of glutathione in a cell, and is preferably a substance that increases glutathione, such as glutathione itself, an enzyme for synthesis of glutathione, and a polynucleotide encoding the enzyme.

The substance for regulating an oxidation-reduction state of a cell can be classified into (i) a substance that can be absorbed into a plant by having contact with the plant and (ii) a substance that is introduced into genome of the plant. It will be understood that these substances can be used singularly or in combination.

The substance that affects synthesis of glutathione or an amount of glutathione and can be absorbed into a plant by having contact with the plant may be, for example, glutathione, glutathione conjugate, active oxygen (hydrogen peroxide, for example), active nitrogen, polyamine, oxidized titanium, jasmonic acid, salicylic acid, cysteine, cystine, heavy-metal cadmium, or iron ion. Polyamine can generate hydrogen peroxide. Oxidized titanium generates active oxygen in response to light. Cysteine and cystine are precursors of glutathione. In regard to heavy-metal cadmium and iron ion, excessive application is preferable. Among the substances exemplified above, glutathione is the most preferable to use. Glutathione includes reduced glutathione (hereinafter referred to as "GSH") and oxidized glutathione (hereinafter referred to as "GSSG"). GSSG is preferable as glutathione to be included in the composition in accordance with the present invention. As described later in Examples, use of GSSG makes it possible to obtain a plant having a higher sugar content. Further, use of GSSG makes it possible to increase the number and size of fruit.

The substance that affects synthesis of glutathione or an amount of glutathione and is introduced into genome of a plant may preferably be, for example, $\gamma$-glutamylcysteine synthetase, a polynucleotide encoding the $\gamma$-glutamylcysteine synthetase (hereinafter referred to as "GSH1 gene"), glu-tathione-binding plastid type fructose-1,6-bisphosphate aldolase, or a polynucleotide encoding the glutathione-binding plastid type fructose-1,6-bisphosphate aldolase (hereinafter referred to as "FBA gene").

Concrete examples of the GSH1 gene are not particularly limited, but include genes of, for example, Zinnia elegans (Genbank accession: AB158510), Oryza sativa (Genbank accession: AJ508915), and Nicotiana tabacum L. (Genbank accession: DQ 444219 ). The genes of these plants can be suitably used in the present invention. Each translation product of these genes has a chloroplast transit signal peptide at its N-terminal region, like Arabidopsis thaliana.

In this regard, however, the following examples (a) through (d) are preferably used as the GSH1 gene in the present invention:
(a) a polynucleotide encoding a polypeptide which has the amino acid sequence of SEQ ID NO: 1 or 3;
(b) a polynucleotide encoding an polypeptide which has a $\gamma$-glutamylcysteine synthetase activity and has an amino acid sequence with deletion, substitution, or addition of one or several amino acids in the amino acid sequence of SEQ ID NO: 1 or 3;
(c) a polynucleotide having the base sequence of SEQ ID NO: 2 or 4; and
(d) a polynucleotide which hybridizes under a stringent condition with a polynucleotide having a base sequence complementary to any one of the polynucleotides of the examples (a) through (c).
Note that the sequence of SEQ ID NO: 2 is an example of a base sequence encoding a polypeptide which has the amino acid sequence of SEQ ID NO: 1 . Note also that the sequence of SEQ ID NO: 4 is an example of a base sequence encoding a polypeptide which has the amino acid sequence of SEQ ID NO: 3.

The FBA gene is not particularly limited, but may preferably be the following examples (e) through (h):
(e) a polynucleotide encoding a protein which has the amino acid sequence of any one of SEQ ID NO: 5, 6, and 15 through 36;
(f) a polynucleotide encoding a protein which has an activity of glutathione-binding plastid type fructose-1,6-bisphosphate aldolase and has an amino acid sequence with deletion, substitution, or addition of one or several amino acids in the amino acid sequence of any one of SEQ ID NO: 5, 6, and 15 through 36;
(g) a polynucleotide having the base sequences of SEQ ID NO: 7 and 37 through 56; and
(h) a polynucleotide which hybridizes under a stringent condition with a polynucleotide having a base sequence complementary to any one of the polynucleotides of the examples (e) through (g).
The sequence of SEQ ID NO: 8 shows a DNA sequence of a protein having the amino acid sequence of SEQ ID NO: 5 . In the base sequence of SEQ ID NO: 8 , the sequence from position 145 to position 147 is a start codon, and the sequence from position 1318 to position 1320 is a stop codon. That is to say, an Arabidopsis thaliana FBA1 gene has the sequence from position 145 to position 1320 of the base sequence of SEQ ID NO: 8 as an open reading frame (ORF).

The sequence of SEQ ID NO: 9 shows an example of a base sequence encoding a protein which has the amino acid sequence of SEQ ID NO: 6. In the sequence of SEQ ID NO: 9 , the sequence from position 104 to position 1300 is a region encoding the protein which has the amino acid sequence of SEQ ID NO: 6. Note that a peptide constituted by amino acids between methionine at position 1 and alanine at position 48 of the sequence of SEQ ID NO: 6 is a chloroplast transit peptide.

The base sequence of SEQ ID NO: 7 is a base sequence serving as an ORF in the Arabidopsis thaliana FBA1 gene. The base sequence of the Arabidopsis thaliana FBA1 gene is homologous with, for example, a gene (dbj|BAB55475.1) found on genome of Oryza sativa.

The sequences of SEQ ID NO: 37 through 56 are examples of DNA sequences encoding the amino acid sequences of SEQ ID NO: 15 through 34, respectively.

For reference, FIG. 9 shows a dendrogram of the amino acid sequences of SEQ ID NO: 15 through 36.

Persons skilled in the art can easily understand that, in a case where the above-mentioned amino acid sequences or DNA sequences include a region corresponding to a chloroplast transit signal, the region can be substituted by a chloroplast transit signal of another protein.

The wording "deletion, substitution, or addition of one or several amino acids" herein means deletion, substitution, or addition of such a number of amino acid(s) (preferably 10 or less, more preferably 7 or less, further preferably 5 or less) that can be deleted, substituted, or added by means of a known method for producing a mutant peptide, such as a site-specific mutation induction method. Such a mutant protein is not limited to a protein which is artificially mutated by means of
a known method for producing a mutant polypeptide, but may be a naturally-existing protein being isolated and purified.

It is known in the art that some amino acids in an amino acid sequence of a protein can be easily altered without significantly affecting a structure or function of the protein. It is also known in the art that a protein has a naturally-existing mutant which does not significantly change a structure or function of the protein, apart from an artificially-altered protein.

It is preferable that a mutant includes conservative or nonconservative substitution, deletion, or addition of amino acid(s). In this regard, silent substitution, addition, and deletion are more preferable, and conservative substitution is particularly preferable. Such mutations do not change a polypeptide activity in accordance with the present invention.
It is considered that representative examples of the conservative substitution are: substitution of one amino acid with another among aliphatic amino acids Ala, Val, Leu, and Ile; replacement of hydroxyl residues Ser and Thr; replacement of acidic residues Asp and Glu; substitution between amide residues Asn and Gln; replacement of basic residues Lys and Arg; and substitution between aromatic residues Phe and Tyr.

The "stringent condition" in the present specification means such a condition that sequences hybridize with each other only when the sequences have at least $90 \%$ identity, preferably at least $95 \%$ identity, most preferably $97 \%$ identity. Specifically, the "stringent condition" includes, for example, incubation overnight at $42^{\circ} \mathrm{C}$. in a hybridization solution ( $50 \%$ formamide, $5 \times$ SSC ( 15 mM trisodium citrate and 150 mM NaCl$), 50 \mathrm{mM}$ sodium phosphate ( pH 7.6 ), $5 \times$ Denhardt's solution, $10 \%$ dextran sulfate, and $20 \mu \mathrm{~g} / \mathrm{ml}$ denatured fragmented salmon sperm DNA) and washing of a filter in $0.1 \times \mathrm{SSC}$ at approximately $65^{\circ} \mathrm{C}$. The hybridization can be carried out by means of a known method such as one described in Sambrook et al., Molecular cloning, A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory (2001). Generally, the higher the temperature is and the lower the salt concentration is, the higher the stringency becomes (the hybridization becomes more difficult to occur). The higher stringency makes it possible to obtain a polynucleotide with a higher homology.

In a case where the composition in accordance with the present invention includes a polynucleotide among the above-mentioned polynucleotides, the composition in accordance with the present invention may include an expression vector including the polynucleotide. The expression vector may be constructed with a known method and is not particularly limited in construction method.

It is possible to use various known vectors as a base of the expression vector. For example, a plasmid, a phage, a cosmid, or the like can be used and selected as appropriate according to an introduction method or a plant cell into which the expression vector is introduced. Specifically, a pBR322 vector, a pBR325 vector, a pUC19 vector, a pUC119 vector, a pBl luescript vector, a pB luescriptSK vector, a pBI vector, or the like can be used, for example. In particular, it is preferable to use a pBI binary vector in a case where the composition in accordance with the present invention is used in introducing a vector that includes the polynucleotide into a plant body by means of the Agrobacterium method. Specifically, the pBI binary vector may be pBIG, pBIN19, pBI101, pBI121, pBI221, or the like, for example.
In the expression vector, a promoter is not particularly limited as long as being able to express a gene in the plant body, and a known promoter can be suitably used. The promoter may be, for example, a cauliflower mosaic virus 35 S promoter (CaMV35S), an actin promoter, a nopaline syn-
thetase promoter, a tobacco PR1a gene promoter, a tomato ribulose-1,5-bisphosphate carboxylase/oxydase small subunit promoter, or the like. Among these promoters, the cauliflower mosaic virus 35 S promoter or the actin promoter can be preferably used. The expression vector with each of the promoters can strongly express a given gene when introduced into a plant cell.

The promoter only has to be introduced into the vector so as to be connected so that a gene encoding a transcription factor can be expressed. The promoter is not particularly limited in specific structure as the expression vector.

The expression vector may further include a DNA segment in addition to the promoter and the polynucleotide. The DNA segment is not particularly limited and may be a terminator, a selection marker, an enhancer, a base sequence for increasing translation efficiency, and the like. Further, the expression vector may include a T-DNA region. The T-DNA region can increase efficiency of gene introduction particularly in a case where the expression vector is introduced into a plant body by means of Agrobacterium.

The terminator is not particularly limited as long as having a function as a transcription termination site, and may be a known terminator. Specifically, it is possible to preferably use a transcription termination site of a nopaline synthetase gene (Nos terminator), a transcription termination site of a cauliflower mosaic virus 35S (CaMV35S terminator), or the like, for example. Among these, the Nos terminator can be more preferably used. By arranging the terminator at an appropriate site in the expression vector, it becomes possible to prevent, after introduction of the expression vector into a plant body, such phenomena that an unnecessarily-long transcript is synthesized and that a strong promoter decreases the number of plasmid copies.

The selection marker may be a drug resistance gene, for example. The drug resistance gene is, for example, one resistant to hygromycin, bleomycin, kanamycin, gentamycin, chloramphenicol, or the like. With the drug resistance gene, it is possible to easily select a transformed plant by cultivating plant bodies in a culture medium that includes the abovementioned antibiotic and thereafter selecting a plant body that can grow in the culture medium.

The polynucleotide for increasing translation efficiency may be, for example, an omega sequence derived from a tobacco mosaic virus. By arranging the omega sequence in an untranslated region ( $5^{\prime} \mathrm{UTR}$ ) of a promoter, it is possible to increase translation efficiency of the gene encoding a transcription factor. As described above, various DNA segments can be included in the expression vector according to purposes.

Specifically, the expression vector is constructed by, for example, a method which the promoter, the polynucleotide, and the DNA segment, if necessary, are introduced into a base vector which is selected accordingly, so as to be arranged in a predetermined order. The polynucleotide and the promoter (and the terminator and the like, if necessary) can be connected so that an expression cassette is constructed, and the expression cassette can be introduced into the base vector. When constructing the expression cassette, it is possible to arrange so that, for example, each DNA segment includes a cleavage site as a protruding end that is complementary to a protruding end of other DNA segment, and these protruding ends are reacted via a ligation enzyme. This makes it possible to regulate an order of the DNA segments. In a case where the terminator is included in the expression cassette, the promoter, a polynucleotide encoding N -acetylglucosamine transferase, and the terminator are arranged in this order from the upstream. Reagents used in constructing the expression
vector, i.e., restriction enzymes, ligation enzymes, and the like, are not particularly limited in type, and commercially available reagents can be accordingly selected and used.

The expression vector can be multiplied by a known method and a multiplication method (production method) of the expression vector is not particularly limited. In general, the expression vector is multiplied in Escherichia coli serving as a host. In this case, a type of $E$. coli can be selected as appropriate according to a type of the expression vector.

It is possible to singularly use the substances exemplified above and to use two or more kinds of the substances in combination.

In a case where the composition in accordance with the present invention includes, as a substance for regulating an oxidation-reduction state of a cell, a substance that can be absorbed into a plant by having contact with the plant, an amount of the substance is not particularly limited, but is preferably 0.01 mM to 20 mM , more preferably 0.1 mM to 2 mM . When the amount of the substance is within the range, it is possible to better improve a sugar content of the plant to be produced. It should be noted that the concentration of the substance may be changed as appropriate according to a desired sugar content, a type of the plant to which the substance is applied, and the like.

The composition in accordance with the present invention may include other component to such an extent that an effect of the composition in accordance with the present invention is not impaired. For example, in a case where the composition in accordance with the present invention includes, as a substance for regulating an oxidation-reduction state of a cell, a substance that can be absorbed into a plant by having contact with the plant, the composition may be dissolved in water, a known liquid carrier, or the like so as to be provided in the form of a liquid agent, an emulsion, a gel agent, or the like. Such a liquid carrier may be, for example, aromatic hydrocarbon such as xylene; alcohol such as ethanol and ethylene glycol; ketone such as acetone; ether such as dioxane and tetrahydrofuran; dimethylformamide, dimethylsulfoxide, acetonitrile, and the like, but is not limited to these. Alternatively, the substance for regulating an oxidation-reduction state of a cell may be supported by a solid carrier component so that the composition is provided as a solid agent, a powder agent, or the like. Such a solid carrier component may be, for example, an inorganic material such as talc, clay, vermiculite, diatomite, kaolin, calcium carbonate, calcium hydroxide, white clay, and silica gel; and an organic material such as flour and starch, but is not limited to these. Further, the composition in accordance with the present invention may be combined with other auxiliary agent accordingly. Such an auxiliary agent may be, for example, an anion surface-active agent such as alkyl sulfate, alkyl sulfonate, alkyl aryl sulfonate, dialkyl sulfosuccinate; a cationic surface-active agent such as higher aliphatic amine salt; a nonionic surface-active agent such as polyoxyethylene glycol alkyl ether, polyoxyethylene glycol acyl ester, polyoxyethylene glycol polyalcohol acyl ester, and cellulose derivative; a thickening agent such as gelatin, casein, and gum arabic; a weighting agent; a binding agent; and the like.

Usage of the composition in accordance with the present invention is not particularly limited. For example, in a case where the composition in accordance with the present invention includes, as a substance for regulating an oxidationreduction state of a cell, a substance that can be absorbed into a plant by having contact with the plant, and where the composition is a liquid agent or the like, the composition may be included in a culture medium or the like which is used in cultivation of the plant, or may be sprayed, dropped, or
applied to entire plant body or a part of the plant body such as a vegetative point, a bud, a leaf, and a stem. Note that a "culture medium" used in cultivation of a plant in the present specification includes soil and a soil improvement agent.

In a case where the composition is a solid agent or the like, the composition may be included in a culture medium which is used in cultivation of a plant. Alternatively, in a case of hydroponic cultivation, the composition may be added to water and gradually dissolved therein. The composition may be applied as a solid agent or the like to be dissolved in water, and dissolved in water at the time of use. Further, the composition in accordance with the present invention may be applied to a plant as a mixture with a known fertilizer and an agent such as a plant hormone.

The composition in accordance with the present invention is not particularly limited in timing of application to a plant. For example, the composition may be applied to the plant from the time of sowing. Specifically, in a case where the composition is applied to a plant such as Lycopersicum esculentum which produces fruit approximately 2 months to half year after sowing, the composition may be applied on the day of sowing and preferably applied in regular intervals during 30 days after sowing, more preferably during 60 days after sowing, further preferably from the day of sowing to the day of harvest. In this case, an interval of application of the composition is not particularly limited, but is preferably one to four times a week, more preferably two or three times a week. The composition is not particularly limited in applied amount. The applied amount can be arranged as appropriate according to a type of plant. In a case of Lycopersicum esculentum or the like, for example, preferably 0.001 mmol or more and 0.1 mmol or less, more preferably 0.01 mmol or more and 0.05 mmol or less, of the substance for regulating an oxidation-reduction state of a cell is applied at a time per plant. In a case where the composition is included in a culture medium as described above, the composition is applied to a plant from the time when the plant is sowed in the culture medium or the time when a seedling or the like of the plant is transplanted to the culture medium.

The composition in accordance with the present invention may be applied to a plant after sowing and after the plant is grown to some extent, e.g., after a seedling of the plant is produced. For example, in a case where the composition is applied to a Gramineae plant such as Zea mays L. var. saccharata Sturt, the composition may be applied to the plant after a seedling of the plant is grown. In this case, the composition in accordance with the present invention may be included in advance in a culture medium to which the seedling is to be transplanted, or may be periodically applied to the culture medium after the seedling is transplanted to the culture medium. In a case where the composition is applied after transplanting of the seedling, timing of the application is not particularly limited. However, it is preferable that, for example, the composition is applied one to four times a week, more preferably two or three times a week, from transplanting of the seeding until harvest. The composition in accordance with the present invention is not particularly limited in applied amount. The applied amount can be arranged as appropriate according to a type of plant. In a case of Zea mays L. var. saccharata Sturt or the like, for example, preferably 0.001 mmol or more and 0.1 mmol or less, more preferably 0.01 mmol or more and 0.05 mmol or less, of the substance for regulating an oxidation-reduction state of a cell is applied at a time per plant.

It is also possible to arrange timing of application of the composition in view of timing of flower production. For example, the composition may be applied while a flower bud
is unbroken, after petals are fallen, from a period that the flower bud is unbroken until fruit bearing, from flowering time until fruit bearing, or from when the petals are fallen until fruit bearing. In a case where the composition is applied to Vitis labrusca as described later in Example, the composition may be applied to anthotaxy. In this Example, the composition is mixed with a plant hormone (gibberellin), which is for producing seedless fruit of Vitis labrusca, and applied when the plant hormone should be applied.

It is also possible to arrange timing of application of the composition based on back calculation of days from harvest time. For example, the composition may be applied 10 days or 20 days before harvest.
In a case where the composition in accordance with the present invention is applied to a plant during cultivation of the plant as described above, the composition may be mixed with a fertilizer and/or an agent such as a plant hormone as described above. In this case, timing of application of a mixture of the composition and the fertilizer or the like is not particularly limited, and the mixture may be applied at a time exemplified above or at a preferable time to apply the fertilizer or the like.

In a case where the composition in accordance with the present invention includes, as a substance for increasing glutathione in a cell, a substance to be introduced into genome of a plant, such as a polynucleotide described above, the composition may be used in such a way that the polynucleotide is introduced into the genome of the plant body by means of a known transformation method. For example, the composition may include a polynucleotide and may be introduced into a plant body by a known plant expression vector, or may include a vector that includes the polynucleotide.

The polynucleotide content of the composition in accordance with the present invention is not particularly limited. The polynucleotide may be dissolved in a buffer or the like which is generally used in polynucleotide preservation.

Introduction of a vector to a plant cell is carried out by a transformation method known in the art (for example, the Agrobacterium method, the particle gun, the polyethylene glycol method, and the electroporation method). In a case of the Agrobacterium method, for example, a constructed plant expression vector is introduced into suitable Agrobacterium (e.g., Agrobacterium tumefaciens) and a aseptically-cultured leaf disc is infected with this strain by the leaf disc method (Hirofumi UCHIMIYA, Manuals for plant genetic manipulation, 1990, 27-31 pp, Kodansha Scientific Ltd., Tokyo) or the like, so that a transformed plant can be obtained. In a case of the particle gun, it is possible to use (i) a plant body, plant organ, or plant tissue without any treatment, (ii) a cut piece of the plant body, plant organ, or plant tissue, or (iii) a protoplast of the plant body, plant organ, or plant tissue. Such a prepared sample can be processed using a gene introduction apparatus (e.g., PDS-1000, Bio-Rad Laboratories, Inc.). In this process, conditions differ according to a plant or a sample, however, are generally arranged so that a pressure is approximately 450 psi to 2000 psi and a distance is approximately 4 cm to 12 cm .

The cell or plant tissue into which a target gene is introduced is selected with a drug-resistance marker such as a kanamycin-resistance marker and a hygromycin-resistance marker, and then reproduced to be a plant body by a standard method. Reproduction of a plant body from a transformed cell can be carried out by a method known in the art according to a type of the plant cell.

In order to determine whether or not a target gene is introduced into a plant, it is possible to use PCR, southern hybridization, northern hybridization, or the like. For example, DNA is prepared from a transformed plant and then subjected
to PCR with use of a primer specific to DNA having been introduced into the transformed plant. Then, an amplification product thus obtained is subjected to agarose gel electrophoresis, polyacrylamide gel electrophoresis, or capillary electrophoresis and thereafter stained with ethidium bromide. As a result, a target amplification product can be detected. In this way, it is possible to determine whether or not the plant is transformed.

Once a transformed plant body in which a target gene is introduced into genome is obtained, it is possible to obtain a progeny of the transformed plant body by sexual or asexual reproduction. Further, it is possible to mass-produce target plant bodies with a reproduction material (e.g., seed, protoplast) obtained from the plant body or the progeny or clone of the plant body.

In the present invention, a target plant for transformation is an entire plant body, a plant organ (for example, leaf, petal, stem, root, and seed), a plant tissue (for example, epidermis, phloem, parenchyma, xylem, vessel bundle, palisade parenchyma, sponge parenchyma), a plant culture cell, a plant cell in various forms (for example, suspension culture cell), protoplast, a cut piece of leaf, callus, or the like. The target plant for transformation is not particularly limited, and a plant capable of expressing a target gene may be selected accordingly.

The polynucleotide mentioned above is derived from Arabidopsis thaliana. It has been reported that, for example, transformed plants of Nicotiana tabacum L., Populus, Citrus limon, and the like can be produced with use of a gene of Arabidopsis thaliana. Such reports also can be used as references for how to use the composition in accordance with the present invention (Franke R, McMichael C M, Meyer K, Shirley A M, Cusumano J C, Chapple C. (2000) Modified lignin in tobacco and poplar plants over-expressing the Arabidopsis gene encoding ferulate 5-hydroxylase. Plant J. 22: 223-234; Pena L, Martin-Trillo M, Juarez J, Pina J A, Navarro L, Martinez-Zapater J M. (2001) Constitutive expression of Arabidopsis LEAFY or APETALA1 genes in citrus reduces their generation time. Nat Biotechnol. 19: 263-267).

Target plants for the composition in accordance with the present invention are not particularly limited. The composition can be applied to almost all plants such as various monocotyledonous plants, dicotyledonous plants, and trees. Examples of monocotyledonous plants include: Lemnaceae such as Spirodela (Spirodela polyrhiza Schleid) and Lemna (Lemna paucicostata and Lemna trisulca); Orchidaceae such as Cattleya, Cymbidium, Dendrobium, Phalaenopsis, Vanda, Paphlopedllum and Oncidium; Typhaceae; Sparganiaceae; Potamogetonaceae; Najadaceae; Scheuchzeriaceae; Alismataceae; Hydrocharitaceae; Triuridaceae; Gramineae (e.g., Zea mays such as Zea mays L. var. saccharata Sturt), Cyperaceae; Palmae; Araceae; Eriocaulaceae; Commelinaceae; Pontederiaceae; Juncaceae; Stemonaceae; Liliaceae; Amaryllidaceae; Dioscoreacea; Iridaceae; Musaceae; Zingiberaceae; Cannaceae; and Burmannia.

Examples of dicotyledonous plants include: Convolvulaceae such as Pharbitis (Pharbitis nil Choisy), Calystegia (Calystegia japonica Choisy, Calystegia hederacea and Calysteegia soldanella Rohm. et Schult.), Ipomoea (Ipomoea pes-caprae and Ipomoea batatas Lam. var. edulis Maikno) and Cuscuta (Cuscuta japonica Chois. and Cuscuta australis); Caryophyllaceae such as Dianthus (Dianthus caryophil$l u s$ L.), Stellaria, Minuartia, Cerastium, Sagina, Arenaria, Moehringia, Pseudostellaria, Hankenya, Spergula, Spergularia, Silene, Lychnis, Melandryum and Cucubalus; Casuarinaceae; Saururacea; Piperaceae; Choranthaceae; Sailicaceae; Myricaceae; Juglandaceae; Betulaceae; Fagaceae;

Ulmaceae; Moraceae; Urticaceae; Podostemaceae; Proteaceae; Olacaceae; Santalaceae; Loranthaceae; Aristolochiaceae; Rafflesiaceae; Balanophoraceae; Polygonaceae; Chenopodiaceae; Amaranthaceae; Nyctaginaceae; Cynocrmbaceae; Phytolaccaceae; Aizoaceae; Portulacaceae; Magnoliaceae; Trochodendraceae; Cercidphyllaceae; Nymphaeaceae; Ceratophyllaceae; Ranunculaceae; Lardizabalaeae; Berberidaceae; Menispermaceae; Calycanthaceae; Lauraceae; Papaveraceae; Capparidaceae; Cruciferae; Droseraceae; Nepenthaceae; Crassulaceae; Saxifragaceae; Pittosporaceae; Hamamelidaceae; Platanaceae; Rosaceae; Leguminosae; Oxalidaceae; Geraniaceae; Linaceae; Zygophyllaceae; Rutaceae; Cimaroubaceae; Meliaceae; Polygalaceae; Euphorbiaceae; Callitrichaceae; Buxaceae; Empetraceae; Coriariaceae; Anacardiaceae; Aquifoliaceae; Celastraceae; Staphyleaceae; Icacinaceae; Aceraceae; Hippocastanaceae; Sapindaceae; Sabiaceae; Balsaminaceae; Rhamnaceae; Vitaceae; Elaeocarpaceae; Tiliaceae; Malvaceae; Stearculiaceae; Actinidiaceae; Theaceae; Guttiferae; Elatinaceae; Tamaricaceae; Violaceae; Flacourtiaceae; Stachyuraceae; Passifloraceae; Begoniaceae; Cactaceae; Thymelaeaceae; Elaegnaceae; Lythraceae; Punicaceae; Rhizophoraceae; Alangiaceae; Melastomataceae; Hydrocaryaceae; Oenotheraceae; Haloragaceae; Hippuridaceae; Araliaceae; Umbelliferae; Cornaceae; Diapensiaceae; Clethraceae; Pyrolaceae; Uricaceae; Myrsinaceae; Primulaceae; Plumbaginaceae; Ebenaceae; Symplocaceae; Styracaceae; Oleaceae; Loganiaceae; Gentianaceae; Apocynaceae; Asclepiadaceae; Polemoniaceae; Boraginaceae; Verbenaceae; Labiatae; Solanaceae (e.g., Lycopersicum esculentum); Scrophulariaceae; Bignoniaceae; Pedaliaceae; Orobanchaceae; Gesneriaceae; Lentibulariaceae; Acanthaceae; Myoporaceae; Phrymaceae; Plantaginaceae; Rubiaceae; Caprifoliaceae; Adoxaceae; Valerianaceae; Dipsacaceae; Cucurbitaceae; Campanulaceae; and Compositae.
The present invention includes a kit for producing a plant body having an improved sugar content (hereinafter referred to as "kit in accordance with the present invention"). The kit in accordance with the present invention only has to include a substance for regulating an oxidation-reduction state of a cell (for example, glutathione, a polynucleotide encoding $\gamma$-glutamylcysteine synthetase, or a polynucleotide encoding glutathione-binding plastid type fructose-1,6-bisphosphate aldolase). Further, the kit in accordance with the present invention may include a component other than the substance above. The substance for regulating an oxidation-reduction state of a cell and the component may be provided together in a single container for containing the substance and the component of an appropriate amount and/or in an appropriate form, or may be separately provided in different containers. Further, the kit in accordance with the present invention may include an instrument for plant cultivation, a culture medium, and the like. In a case where a polynucleotide is included in the kit in accordance with the present invention, the kit may be such that a base vector of an expression vector for expressing the polynucleotide may be provided in a different container from the polynucleotide. Alternatively, the kit may include the base vector into which the polynucleotide is introduced in advance. Further, the kit in accordance with the present invention may include a reagent and the like which is used in a known plant transformation method.

## 2. Method, in Accordance with the Present Invention, for Producing Plant Body Having Improved Sugar Content

A method, in accordance with the present invention, for producing a plant body having an improved sugar content
(hereinafter referred to as "method in accordance with the present invention") only has to include a step for cultivating a plant body with use of a substance for regulating an oxida-tion-reduction state of a cell (for example, glutathione, a polynucleotide encoding $\gamma$-glutamylcysteine synthetase, or a polynucleotide encoding glutathione-binding plastid type fructose-1,6-bisphosphate aldolase).

In a case where a substance that can be absorbed into a plant by having contact with the plant is used in regulation of an oxidation-reduction state of a cell, the step may include, for example, causing the plant to absorb the substance. How to cause the plant to absorb the substance for regulating an oxidation-reduction state of a cell is not particularly limited. For example, it is possible to cause the plant to absorb the substance by cultivating the plant on a culture medium (including soil and an soil improvement agent) that includes the substance, or by spraying or coating the plant with the substance during cultivation of the plant. Alternatively, it is also possible to cultivate the plant on a culture medium that includes absorbent such as an ion-exchange resin into which the substance is absorbed, where the absorbent is buried in soil of the culture medium, for example.

In a case where a substance such as a polynucleotide which is to be introduced into genome of a plant is used in regulation of an oxidation-reduction state of a cell, the method does not include causing the plant to absorb the substance, but may include introducing the substance to the plant in advance so as to produce a transformed plant and then cultivating the transformed plant. How to introduce a polynucleotide into the plant is described above in the explanation of the composition in accordance with the present invention.

The present invention includes a plant body obtained by the method in accordance with the present invention. It is possible to easily identify the plant body by measuring at least either a content or ratio, in the plant body, of the substance for regulating an oxidation-reduction state of a cell. Therefore, it is possible to clearly distinguish the plant body from one obtained by other method. The plant body can be identified also by, for example, comparing gene expression patterns by means of DNA microarray or the like, other than by measuring the content and concentration of the substance. In a case where GSSG is used as the substance, it is possible to take the following procedures, for example: (i) a gene expression pattern of a plant cultivated after being applied with GSSG is analyzed in advance; (ii) an expression pattern unique to the plant body applied with GSSG (GSSG expression pattern) is determined by comparison of gene expression pattern between the plant body applied with GSSG and a plant body cultivated by other method; (ii) an expression pattern of a target plant body is analyzed; and then (iv) the expression pattern of the target plant body is compared with the GSSG expression pattern. This allows an easy identification of the plant body applied with GSSG. Further, as another example of the identification, comparison of a two-dimensional electrophoretic profile of a glutathione-binding protein to a pattern change analyzed in advance makes it possible to determine whether or not GSSG is applied. In a case where a polynucleotide is used, it is possible to distinguish the plant body in accordance with the present invention from other plant body by identifying the polynucleotide in the plant body by means of PCR, southern hybridization, northern hybridization, or the like.

Details of the embodiments of the present invention are described below in Examples. It will be obvious that the present invention is not limited to the descriptions of the examples below and details of the present invention may be varied in many ways. The present invention is not limited to
the description of the embodiments above, but may be altered by a skilled person within the scope of the claims. An embodiment based on a proper combination of technical means disclosed in different embodiments is encompassed in the technical scope of the present invention. All documents cited is incorporated herein by reference.

## EXAMPLES

## Example 1

## Production of Lycopersicum esculentum

In the present example, Lycopersicum esculentum was cultivated with use of GSSG or GSH. Details of cultivation are described below.
First, Lycopersicum esculentum seedlings (TAKII \& CO. Ltd., product name: Osama tomato reika) were transplanted into a hydroponic culture pot ( $1 / 2000 \mathrm{a}$ ). In the hidroponic culture pot, 6 L of vermiculite (ASAHI INDUSTRIES Co., LTD.), 3 L of KUREHA horticultural soil (KUREHA CORPORATION), and 3 L of vermiculite were layered as a lower, middle, and upper layers, respectively.

During the cultivation of Lycopersicum esculentum, 50 mL of 0.5 mM GSSG or 0.5 mM GSH (adjusted with 0.1 N NaOH to be at pH 7 ) was applied twice a week at a root per plant. The Lycopersicum esculentum plants were grown for 60 days without being subjected to bud removal. Last 10 days was used as a harvest period for harvesting fruit of the plants. For comparison, a Lycopersicum esculentum plant was grown under the same condition, except that GSSG and GSH were not applied. To the plants of any condition, 3 g of Kumiai phosphorate ammonium nitrate potassium S-604 (Chisso Asahi Fertilizer Co., Ltd.) was applied as an additional fertilizer once in 2 weeks.

Next, the fruit harvested was subjected to sensory tests of sugar content and the like. As a result, it was determined that fruit of the plant applied with GSSG increased in sugar content compared to that of the plant not applied with GSSG or GSH. Further, it was determined that the plant applied with GSSG increased in number of fruit. It was determined that fruit of the plant applied with GSH increased in sugar content and acidity.

These results indicated that Lycopersicum esculentum having an increased sugar content could be produced by cultivation using a culture medium that contains GSSG or GSH.

## Example 2

## Sugar Content Determination

Cultivated were Lycopersicum esculentum plants to which GSSG or GSH was applied by the method described in Example 1. Then, obtained fruit of the plants was subjected to sugar content determination using "Pocket" Refractometer APAL-1 (ATAGO CO., LTD.).

For comparison, Lycopersicum esculentum plants were cultivated under two types of conditions (referred to as "Cont" and "Cont2 Sunny"). In the Cont condition, Lycopersicum esculentum plants were cultivated by the same method as in Example 1, except that GSSG and GSH were not applied. In the Cont2 Sunny condition, a Lycopersicum esculentum plant was not applied with GSSG or GSH and was independently cultivated at a site sufficiently irradiated with sunlight so that illuminance on the Lycopersicum esculentum plant becomes $100 \%$. In the Cont condition and a condition in which GSSG or GSH was applied, the plants were planted at
intervals of 40 cm to 50 cm . In this case, a plant may intercept light irradiating another plant. Therefore, illuminance on such plants becomes less than $100 \%$.

In the condition in which GSSG was applied, the condition in which GSH is applied, and the Cont condition, three Lycopersicum esculentum plants were cultivated, respectively. In the Cont2 Sunny condition, one Lycopersicum esculentum plant was cultivated.

FIGS. 1 and 2 show results of the sugar content determination. FIG. 1 shows a result of sugar content determination of Lycopersicum esculentum plants obtained in the present example. In FIG. 1, the vertical scale indicates sugar content (Brix, unit: \%) and the horizontal scale indicates cultivation conditions. In FIG. 1, the reference sign * indicates that fruit could not be obtained during the harvest period. FIG. 2 shows a result of ANOVA analysis on the result of sugar content determination shown in FIG. 1. In FIG. 2, the vertical scale indicates sugar content and the horizontal scale indicates cultivation conditions. In FIG. 2, alphabetic characters above each bar are for indicating that bars indicated by a same character belong to a same group when being grouped based on ANOVA analysis. The ANOVA analysis was carried out by means of StatView 5.0 (SAS Institute Inc.) with a significant difference level of $5 \%$.

As shown in FIGS. 1 and 2, application of GSSG or GSH made it possible to obtain Lycopersicum esculentum fruit which was significantly increased in sugar content compared to Lycopersicum esculentum fruit cultivated under the Cont condition and also to Lycopersicum esculentum fruit sufficiently irradiated with sunlight. Especially, application of GSSG made it possible to obtain Lycopersicum esculentum having an extremely high sugar content.

## Example 3

## Production of Zea mays L. var. saccharata Sturt

In the present example, Zea mays L. var. saccharata Sturt was cultivated. First, a Zea mays L. var. saccharata Sturt seed (TAKII \& CO. Ltd., product number: Canberra 90) was sown in vermiculite (ASAHI INDUSTRIES Co., LTD.). Two weeks after sowing, a Zea mays L. var. saccharata Sturt plant was transplanted to a hydroponic culture pot described in Example 1. To the plant, 3 g of Kumiai phosphorate ammonium nitrate potassium S-604 (Chisso Asahi Fertilizer Co., Ltd.) was applied as an additional fertilizer 4 weeks and 6 weeks after the sowing.

Within 2 weeks from the 5 th week after the sowing, 50 mL of 0.2 mM GSSG was applied 4 times at a root of the plant. Within 2 weeks from the 7 th week after the sowing, 50 mL of 0.2 mM GSSG was sprayed 4 times to leaves of the plant. For comparison, a Zea mays L. var. saccharata Sturt plant was cultivated by the same method as in the present example, except that GSSG was not applied, and fruit thereof was harvested.

Fruit was harvested 90 days after the sowing and subjected to a sensory test of sugar content. As a result, it was determined that fruit of the plant applied with GSSG increased in sugar content compared to that of the plant applied with no GSSG. Further, it was determined that the plant applied with GSSG increased in size and number of fruit.

## Example 4

> Production of Zea mays L. var. saccharata Sturt (2)

In the present example, Zea mays L. var. saccharata Sturt was cultivated under a condition different from Example 3 in
how to apply GSSG. First, a Zea mays L. var. saccharata Sturt seed (TAKII \& CO. Ltd., product number: Canberra 90) was sown in vermiculite (ASAHI INDUSTRIES Co., LTD.). One week after sowing, a Zea mays L. var. saccharata Sturt plant was transplanted to a hydroponic culture pot described in Example 1. To the plant, 3 g of Kumiai phosphorate ammonium nitrate potassium S-604 (Chisso Asahi Fertilizer Co., Ltd.) was applied as an additional fertilizer 4 weeks and 6 weeks after the sowing.
During 12 weeks after germination, 200 mL of 0.5 mM GSSG was applied at a root of the plant twice a week. For comparison, a Zea mays L. var. saccharata Sturt plant was cultivated by the same method as in the present example, except that GSSG was not applied, and fruit thereof was harvested.

Fruit was harvested 12 weeks after the sowing and subjected to a sensory test of sugar content. As a result, it was determined that fruit of the plant applied with GSSG increased in sugar content compared to that of the plant applied with no GSSG. Further, it was determined that the plant applied with GSSG increased in size and number of fruit.

## Example 5

## Production of Vitis labrusca

In the present invention, Vitis labrusca was cultivated. Specifically, immediately after flowering of a Vitis labrusca (Delaware) plant, a mixed solution of 1 mM gibberellin (GA3) and 1 mM of an agent was applied to anthotaxy of the plant. The agent was GSSG or GSH. Then, the plant was coated with the agent and thereafter produced fruit was harvested. For comparison, a Vitis labrusca plant was cultivated in the same way, except that GA3, but not GSSG or GSH, was applied, and fruit thereof was harvested and subjected to a sensory test described below.

The fruit harvested was subjected to a sensory test of sugar content. As a result, it was determined that fruit of the plant applied with GA3 and GSSG or GSH increased in sugar content compared to that of the plant applied with only GA3. Further, it was determined that the plant applied with GSSG and GA3 increased in size of fruit.

In addition, it was determined that a Vitis labrusca plant applied with GSSG or GSH but not GA3 increased in sugar content. In this case, effect of producing seedless grape was suppressed without GA3.

## Example 6

Change Over Time after Application of Substance for Regulating Oxidation-Reduction State of Cell

In the present example, a sugar content of a plant was determined after a substance for regulating an oxidationreduction state of a cell was applied to the plant. The substance for regulating an oxidation-reduction state of a cell was GSH or GSSG. As in the case of Example 1, Lycopersicum esculentum was used as the plant. Specifically, the following operations were carried out.

Ninety days after sowing of Lycopersicum esculentum seeds, Lycopersicum esculentum plants were subjected to a GSH or GSSG treatment. The Lycopersicum esculentum plants were cultivated by the same method as in Example 1 except for the GSH or GSSG treatment. The GSH or GSSG treatment was such that 50 mL of 0.5 mM GSSH or 0.5 mM GSH (adjusted with 0.1 N NaOH to be at pH 7 ) was applied
once at a root per plant. Then, fruit of the plants was harvested every day from the 0th day until the 4th day after application of GSH or GSSG, and subjected to sugar content determination. FIG. 3 shows a result of the sugar content determination. FIG. $\mathbf{3}$ is a graph showing a determination result of relation between sugar content and the number of days from an application day of GSH or GSSG. In FIG. 3, the vertical scale indicates sugar content (Brix, unit: \%) and the horizontal scale indicates days from the application day. In FIG. 3, lines labeled with circles, triangles, and squares show results of the plants applied with GSH, GSSG, and no GSH and no GSSG, respectively. Note that GSSG or GSH was applied in the morning of the 0th day, and a result of the 0th day in FIG. 3 was obtained by harvesting fruit and determining a sugar content of the fruit in the evening of the 0th day.

As shown in FIG. 3, it was shown that application of GSSG or GSH made it possible to rapidly improve a sugar content of fruit.

## Example 7 <br> Production of Plant into which GSH1 Gene is Introduced

In the present example, a clone of a $\gamma$-glutamylcysteine synthetase gene was used as a substance for regulating an oxidation-reduction state of a cell. The clone is a polynucleotide having a sequence of SEQ ID NO:3, is one of GSH1 genes, and is referred to merely as "GSH1 gene" in the present example.
(1) Plant to be Used

In order to produce a transformed plant, a wild type Arabidopsis thaliana Columbia (Col-0) was used as a parent plant. The Columbia (Col-0) was sown in soil in a square plastic pot ( $6.5 \times 6.5 \times 5 \mathrm{~cm}$ ), which soil is constituted by three layers of vermiculite (ASAHI INDUSTRIES Co., LTD., Okayama), KUREHA culture soil (KUREHA horticultural soil, KUREHA CORPORATION, Tokyo), and vermiculite being layered in this order from the bottom at a ratio of 2:1:1. Then, the Columbia ( $\mathrm{Col}-0$ ) was cultivated at a growth temperature of $22^{\circ} \mathrm{C}$. under a long-day condition ( 16 -hour light period/8-hour dark period).
(2) Cloning of GSH1 Gene, Alteration of GSH1 Gene, and Production of GSH1-Transformed Plant

Entire RNA of a 3-week-old wild type Arabidopsis thaliana Columbia (Col-0) was isolated, and cDNA was synthesized based on the RNA by using a Prostar first strand RT-PCR kit (Stratagene, La Jolla, Calif., USA).

With use of the following specific primers designed based on a cDNA sequence of a GSH1 gene, a full-length cDNA was amplified as two fragments by PCR:

```
GSH1_5'-3:
5'-GCTTTCTTCTAGATTTCGACGG-3' (SEQ ID NO: 10)
GSH1 3'-3:
5'-CCTGATCATATCAGCTTCTGAGC-3' (SEQ ID NO: 11)
GSH1_5'-2 :
5'-ATGCCAAAGGGGAGATACGA-3' (SEQ ID NO: 12)
GSH1_3'-2:
5'-GGAGACTCGAGCTCTTCAGATAG-3'. (SEQ ID NO: 13)
```

Then, subcloning was carried out so that each of the fragments was inserted into a pGEM-T Easy vector (Promega, Madison, Wis., USA). The primers GSH1 5'-3 and GSH1 $3^{\prime}-2$ respectively includes XbaI and SacI cleavage sites
required for introduction of the fragments to a binary vector pBI121 used in plant transformation.

The two fragments were fused with each other at a KpnI cleavage site, so that a vector (Ch1.GSH1-pGEM) including the full-length cDNA was constructed. The Ch1.GSH1pGEM was treated with restriction enzymes XbaI and SacI and a fragment thus obtained was substituted with a region of a binary vector pBI 121 , which region encodes $\beta$-glucuronidase (GUS) and is located downstream of a cauliflower mosaic virus 35 S promoter. As a result, a construct ( 35 S -Ch1.GSH1-pBI121) for producing the transformed plant was produced.

There is only one copy of the GSH1 gene in genome of Arabidopsis thaliana, and the GSH1 gene includes a chloroplast transit signal. For the purpose of accumulating GSH1 gene products ( $\gamma$-glutamylcysteine synthetase) in cytoplasm, produced was a construct (35S-cyt.GSH1-pBI121) for expressing a protein in which the 73 rd amino acid from an N-terminal, which amino acid was presumed to be the chloroplast transit signal, was deleted and an alanine residue at the $74^{\text {th }}$ position from the N -terminal was substituted with a methionine residue. First, PCR was performed with the primer GSHI_3'-3 and the following primer GSH1 (cyt.)_ $5^{\prime}$ (a base substitution site is underlined) in which the alanine residue at the 74th position from the N-terminal was substituted with the methionine residue and an XbaI cleavage site was inserted upstream of the $74^{\text {th }}$ position:

GSH1 (cyt.)_5':
$5^{\prime}$-AGGGCATCTAGAGACCATGGCAAGTCC-3'. (SEQ ID NO: 14)
Then, a fragment thus obtained was treated with restriction enzymes XbaI and KpnI. Thereafter, subcloning was carried out so that the fragment was inserted into a pBluescript vector (Stratagene, La Jolla, Calif. USA) (cyt.GSH-1pBS). The cyt.GSH1-pBS was treated with the restriction enzymes XbaI and KpnI , and a fragment thus obtained was substituted with a XbaI-KpnI fragment of the 35S-Ch1.GSHI-pBI121. As a result, the 35S-cyt.GSH1-pBI121 was produced.

The two types of expression vectors produced as above, i.e., the 35S-Ch1.GSH1-pBI121 and the 35S-cyt.GSH1$\mathrm{pBI121}$, were introduced into the Col-0 by the Agrobacterium method (Clough, S. J. and SH1-pB Bent, A. F. (1998) Floral dip: A simplified method for Abrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16: 735-743). As a result, a transformed plant was produced.

Specifically, selection of the transformed plant was repeated on an agar medium (Murashige-Skoog medium of a half concentration) which contains kanamycin serving as a selection marker, until such a generation occurred that all seeds exhibit kanamycin resistance (a generation does not exhibit divergence). In process of the selection, it was determined that characters of the kanamycin resistance were diverged at a ratio of $3: 1$ and that the expression vectors were introduced into at least single chromosome.

The plant obtained as above is hereinafter referred to as "35S-GSH1".
(3) Sugar Content Determination

A 35S-GSH1 and a wild type Arabidopsis thaliana (Col-0) for comparison were cultivated at a growth light intensity of $50 \mu \mathrm{Em}^{-2} \mathrm{~s}^{-1}$ or $500 \mu \mathrm{Em}^{-2} \mathrm{~s}^{-1}$. After one-week cultivation, each plant body was collected. Then, each plant body was frozen with liquid nitrogen, ground into powder, and thereafter subjected to extraction using $100 \mu 1$ of 50 mM sodium acetate buffer per 50 mg of plant body.

Next, a glucose content and a starch content of each extract thus obtained were determined. The glucose content was determined using Glucose CII-Test Wako (Wako Pure Chemical Industries, Ltd.). The starch content was determined by mixing the extract with 35 Units $/ \mathrm{ml}$ amyloglucanase and a sodium acetate buffer ( $50 \mathrm{mM}, \mathrm{pH} 4.5$ ), leaving at rest the resulting mixture for 1 hour, and then determining an amount of glucose. Results of determination are shown in FIG. 4. FIG. 4 shows determination results of starch and glucose contents of 35S-GSH1. In FIG. 4, (a) shows starch contents, and (b) shows glucose contents. In (a) and (b) of FIG. 4, the vertical scales indicate relative contents of starch and glucose, respectively, and the horizontal scales indicate types of plants. A and B shown in FIG. 4 are results of the $35 \mathrm{~S}-\mathrm{GSH} 1$. In the present example, two $35 \mathrm{~S}-\mathrm{GSH} 1$ plants were used in an experiment as $A$ and $B$ shown in FIG. 4. The term "relative content" above means a relative amount where an amount in the Col-O cultivated at a growth light intensity of $50 \mu \mathrm{Em}^{-2} \mathrm{~s}^{-1}$ is 100 .

As shown in FIG. 4, the 35S-GSH1 had a higher starch content and a higher sugar content than the Col-0.

## Example 8

## Production of Prunus avium

In the present example, Prunus avium was cultivated. Specifically, 4 weeks and 3 weeks before an expected date of harvesting Prunus avium (Napoleon) fruit, a surface of a leaf on a branch having the fruit to be harvested was coated with 0.5 mM GSSG. The fruit was harvested on the expected date.

Next, the fruit harvested was subjected to a sensory test of sugar content. As a result, it was determined that the fruit applied with GSSG increased in sugar content and decreased in acidity. Further, it was determined that the fruit applied with GSSG increased in weight. Furthermore, the fruit obtained was subjected to sugar content determination using "Pocket" Refractometer APAL-1 (ATAGO CO., LTD.). For comparison, fruit applied with no GSSG was also subjected to the sugar content determination. FIG. 5 shows a result of determination of sugar content of Prunus avium fruit obtained in the present example. In FIG. $\mathbf{5}$, the vertical scale indicates sugar content (Brix, unit: \%). Further, an ANOVA analysis was carried out by using StatView5.0 (SAS Institute Inc.) with a significant difference level of $5 \%$. As a result, a significant difference was shown.

As described above, application of GSSG made it possible to obtain Prunus avium fruit having a significantly improved sugar content.

## Example 9

## Production of Citrus unshiu

In the present example, Citrus unshiu was cultivated. Spe- 55 cifically, one week before an expected date of harvesting Citrus unshiu fruit, a surface of a leaf on a branch having the fruit to be harvested was coated with 0.5 mMGSSG . The fruit was harvested on the expected date.

Next, the fruit harvested was subjected to a sensory test of 60 sugar content. As a result, it was determined that the fruit applied with GSSG increased in sugar content and decreased in acidity. Further, it was determined that the fruit applied with GSSG increased in weight. Furthermore, the fruit obtained was subjected to sugar content determination using "Pocket" Refractometer APAL-1 (ATAGO CO., LTD.). For comparison, fruit applied with no GSSG was also subjected to
the sugar content determination. FIG. 6 shows a result of determination of sugar content of Citrus unshiu fruit obtained in the present example. In FIG. 6, the vertical scale indicates sugar content (Brix, unit: \%). Further, an ANOVA analysis was carried out by using StatView5.0 (SAS Institute Inc.) with a significant difference level of $5 \%$. As a result, a significant difference was shown.
As described above, application of GSSG made it possible to obtain Citrus unshiu fruit having a significantly improved sugar content.

## Example 10

## Production of Fragaria ananassa

In the present example, Fragaria ananassa was cultivated with use of GSSG or GSH. Details of cultivation are described below.
First, Fragaria ananassa seedlings were transplanted to a planter. In the planter, 6 L of vermiculite (ASAHI INDUSTRIES Co., LTD.), 3 L of KUREHA horticultural soil (KUREHA CORPORATION), and 3 L of vermiculite were layered as a lower, middle, and upper layers, respectively.

During cultivation of Fragaria ananassa plants, 50 mL of 0.2 mM or 0.5 mM GSSG or 50 mL of 0.4 mM or 0.5 mM GSH (adjusted with 0.1 N NaOH to be at pH 7 ) was applied once a week at a root per plant. The plants were grown for 63 days without being subjected to bud removal. For comparison, a Fragaria ananassa plant was grown under the same condition, except that GSSG and GSH were not applied. To the plants of any condition, 3 g of Kumiai phosphorate ammonium nitrate potassium S-604 (Chisso Asahi Fertilizer Co., Ltd.) was applied as an additional fertilizer once in 2 weeks.

Next, the fruit harvested was subjected to sensory tests of sugar content and the like. As a result, it was determined that fruit of the plant applied with GSSG increased in sugar content and decreased in acidity compared to that of the plant not applied with GSSG or GSH. Further, it was determined that the plant applied with GSSG increased in number of fruit. It was also determined that fruit of the plant applied with GSH increased in sugar content and acidity.

Further, the fruit obtained was subjected to sugar content determination using "Pocket" Refractometer APAL-1 (ATAGO CO., LTD.). For comparison, fruit not applied with GSSG or GSH was also subjected to the sugar content determination. FIG. 7 shows a result of determination of sugar content of Fragaria ananassa fruit obtained in the present example. In FIG. 7, the vertical scale indicates sugar content (Brix, unit: \%). Further, an ANOVA analysis was carried out by using StatView5.0 (SAS Institute Inc.) with a significant difference level of $5 \%$. As a result, a significant difference was shown.

These results indicated that Fragaria ananassa fruit having an increased sugar content could be produced by cultivation using a culture medium that contains GSSG or GSH.

## Example 11

## Production of Zea mays L. var. saccharata Sturt

In the present example, Zea mays L. var. saccharata Sturt was cultivated. First, a Zea mays L. var. saccharata Sturt seed (TAKII \& CO. Ltd., product number: Canberra 86) was sown in vermiculite (ASAHI INDUSTRIES Co., LTD.). Two weeks after sowing, a Zea mays L. var. saccharata Sturt plant was transplanted to a hydroponic culture pot described in Example 1. To the plant, 3 g of Kumiai phosphorate ammo-
nium nitrate potassium S-604 (Chisso Asahi Fertilizer Co., Ltd.) was applied as an additional fertilizer 4 weeks and 6 weeks after the sowing.

In the 5th, 6 th, 7 th, and 8th week after the sowing, 0.5 mM GSSG (dissolved in $0.1 \%$ Tween80 serving as a spreading agent) was sprayed onto a leaf surface. For comparison, a Zea mays L. var. saccharata Sturt plant was cultivated by the same method as in the present example, except that Tween80, but not GSSG, was applied, and fruit thereof was harvested.

Fruit was harvested 86 days after the sowing and subjected to a sensory test of sugar content. As a result, it was determined that fruit of the plant applied with GSSG increased in sugar content compared to that of the plant applied with no GSSG. Further, the fruit obtained was subjected to sugar content determination using "Pocket" Refractometer APAL-1 (ATAGO CO., LTD.). For comparison, the fruit of the plant applied with no GSSG was also subjected to the sugar content determination. FIG. 8 shows a result of determination of sugar content of Zea mays L. var. saccharata Sturt fruit obtained in the present example. In FIG. 8, the vertical scale indicates sugar content (Brix, unit: \%). Further, an ANOVA analysis was carried out by using StatView 5.0 (SAS Institute Inc.) with a significant difference level of $5 \%$. As a result, a significant difference was shown.

It was also determined that the plant applied with GSSG increased in size and number of fruit. Further, it was determined that the fruit of the plant applied with GSSG was already able to be harvested 70 days after the sowing.

The results above indicated that Zea mays L. var. saccharata Sturt fruit having an increased sugar content could be produced by cultivation using a culture medium that includes GSSG.

The composition, in accordance with the present invention, for producing a plant body having an improved sugar content includes a substance for regulating an oxidation-reduction state of a cell. Therefore, with the composition in accordance with the present invention, it is possible to easily produce the plant body having an improved sugar content.

The embodiments and concrete examples of implementation discussed in the foregoing detailed explanation serve solely to illustrate the technical details of the present invention, which should not be narrowly interpreted within the limits of such embodiments and concrete examples, but rather may be applied in many variations within the spirit of the present invention, provided such variations do not exceed the scope of the patent claims set forth below.

## INDUSTRIAL APPLICABILITY

The composition in accordance with the present invention, with which a plant having an improved sugar content can be easily produced, is industrially applicable in agriculture, food industry, and the like. Further, because ethanol can be produced with high efficiency from a plant having a high sugar content, the composition in accordance with the present invention is applicable to a wide range of industries such as energy industry.

SEQUENCE LISTING


$<210>$ SEQ ID NO 2
$<211>$ LENGTH: 1569
$<212>$ TYPE: DNA
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 2
 OTT SOT OOT




 of $9 Z \quad$ oz
 GT OL G $\quad$ G nəT oxd nโp xप山 ety ten ten ety ntp ntp xul oxd oxd xəs etz コəW


 6もも ：HWゆN＇T＜T．Lて＞ $\varepsilon$ ON aI Öㅍㄴ＜OTZ＞












 7е66е666๐7 6о667ееего จ6еро77о66 77ее66е77о 7776677ее6 667еее66е6









SLT OLT
S9T



0も SعL
$0 \varepsilon \tau$
 GZT 0 ZT STL



 $56 \quad 06 \quad 98$
 08 SL 0L S9


 $0 \varepsilon \quad \mathrm{~s} Z$ OZ
 ST $\qquad$ S T

G：gDNGロṎS＜OOも＞

山甘d ：田dス山＜てIて＞ T6 ：HLDNHT＜T．Z＞











 จ7ขео6ое7๐ е67767е76е จ6е6777666 7777070е67 е607777677 7еоое7067е








|  |  | 180 |  |  |  | 185 |  |  |  |  | 190 |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ala | $\begin{array}{r} \text { Val Lys } \\ 195 \end{array}$ | Glu Ala | Ala |  | $\begin{aligned} & \text { Gly } \\ & 200 \end{aligned}$ | Leu | Ala | Arg | Tyr | $\begin{aligned} & \text { Ala } \\ & 205 \end{aligned}$ |  | Ile Ser |
| Gln | Asp Asn 210 | Gly Leu | Val | $\begin{aligned} & \text { Pro } \\ & 215 \end{aligned}$ | Ile | Val | Glu | Pro | $\begin{aligned} & \text { Glu } \\ & 220 \end{aligned}$ | Ile | Leu | Leu Asp |
| $\begin{aligned} & \text { Gly } \\ & 225 \end{aligned}$ | Asp His | Pro Ile | $\begin{aligned} & \text { Glu } \\ & 230 \end{aligned}$ | Arg | Thr | Leu | Glu | $\begin{aligned} & \text { Val } \\ & 235 \end{aligned}$ | Ala | Glu | Lys | $\begin{aligned} & \text { Val } \text { Trp } \\ & 240 \end{aligned}$ |
| Ser | Glu Val | $\begin{array}{r} \text { Phe Phe } \\ 245 \end{array}$ | Tyr |  | Ala | $\mathrm{Gln}$ | $\begin{aligned} & \text { Asn } \\ & 250 \end{aligned}$ | Asn |  | Met | Phe | $\begin{aligned} & \text { Glu Gly } \\ & 255 \end{aligned}$ |
| Ile | Leu Leu | $\begin{aligned} & \text { Lys Pro } \\ & 260 \end{aligned}$ | Ser | Met | Val | $\begin{aligned} & \text { Thr } \\ & 265 \end{aligned}$ | Pro | Gly | Ala | Glu | $\begin{aligned} & \mathrm{His} \\ & 270 \end{aligned}$ | Lys Asn |
| Lys | $\begin{array}{r} \text { Ala Ser } \\ 275 \end{array}$ | Pro Glu | Thr |  | $\begin{aligned} & \text { Ala } \\ & 280 \end{aligned}$ | Asp | Phe | Thr | Leu | $\begin{aligned} & \text { Thr } \\ & 285 \end{aligned}$ | Met | Leu Lys |
| Arg | $\begin{aligned} & \text { Arg Val } \\ & 290 \end{aligned}$ | Pro Pro | Ala | $\begin{aligned} & \text { Val } \\ & 295 \end{aligned}$ | Pro | Gly | Ile | Met | $\begin{aligned} & \text { Phe } \\ & 300 \end{aligned}$ | Leu | Ser | Gly Gly |
| $\begin{aligned} & \text { Gln } \\ & 305 \end{aligned}$ | Ser Glu | Ala Glu | $\begin{aligned} & \text { Ala } \\ & 310 \end{aligned}$ | Thr | Leu | Asn | Leu | $\begin{aligned} & \text { Asn } \\ & 315 \end{aligned}$ | Ala | Met | Asn | $\begin{array}{r} \text { Gln Ser } \\ 320 \end{array}$ |
| Pro | Asn Pro | $\begin{array}{r} \text { Trp His } \\ 325 \end{array}$ | Val |  | Phe | Ser | $\begin{aligned} & \text { Tyr } \\ & 330 \end{aligned}$ | Ala | Arg | Ala | Leu | $\begin{aligned} & \text { Gln Asn } \\ & 335 \end{aligned}$ |
| Ser | Val Leu | $\begin{aligned} & \text { Arg Thr } \\ & 340 \end{aligned}$ | $\operatorname{Trp}$ | $\mathrm{Gln}$ | Gly | $\begin{aligned} & \text { Lys } \\ & 345 \end{aligned}$ | Pro | Glu | Lys | Ile | $\begin{aligned} & \text { Glu } \\ & 350 \end{aligned}$ | Ala Ser |
| Gln | $\begin{array}{r} \text { Lys Ala } \\ 355 \end{array}$ | Leu Leu | Val | Arg | $\begin{aligned} & \text { Ala } \\ & 360 \end{aligned}$ | Lys | Ala | Asn | Ser | $\begin{aligned} & \text { Leu } \\ & 365 \end{aligned}$ | Ala | Gln Leu |
| Gly | $\begin{aligned} & \text { Lys Tyr } \\ & 370 \end{aligned}$ | Ser Ala | Glu | $\begin{aligned} & \text { Gly } \\ & 375 \end{aligned}$ | Glu | Asn | Glu | Asp | $\begin{aligned} & \text { Ala } \\ & 380 \end{aligned}$ | Lys | Lys | Gly Met |
| $\begin{aligned} & \text { Phe } \\ & 385 \end{aligned}$ | Val Lys | Gly Tyr | $\begin{aligned} & \text { Thr } \\ & 390 \end{aligned}$ | Tyr |  |  |  |  |  |  |  |  |

$<210>$ SEQ ID NO 6
$<211>$ LENGTH: 398
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 6





























 ZNQ ：島dXL＜てT己＞ GTGT ：HLDNGT＜TTZ＞ 8 ON aI ÖNS＜OL己＞






006


## pənuț ユuoŋ－



：버배볍＜0Z乙＞

चNG ：出机＜ZTZ＞
とて ：HWゆN＇T＜TTて＞
t．ON aI ṎS＜OTZ＞

```
<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<400> SEQUENCE: 12
```

atgccaaagg ggagatacga
$<210>$ SEQ ID NO 13
$<211>$ LENGTH: 23
$<212>$ TYPE : DNA
$<213>$ ORGANISM: Artificial
$<220>$ FEATURE:
$<223>$ OTHER INFORMATION: Primer
$<400>$ SEQUENCE: 13
ggagactcga getcttcaga tag
$<210>$ SEQ ID NO 14
$<211>$ LENGTH: 27
$<212>$ TYPE: DNA
$<213>$ ORGANISM: Artificial
$<220>$ FEATURE:
$<223>$ OTHER INFORMATION: Primer
$<400>$ SEQUENCE: 14
agggcatcta gagaccatgg caagtcc
$<210>$ SEQ ID NO 15
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 15


$<210>$ SEQ ID NO 16
$<211>$ LENGTH: 352
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 16

| $\begin{aligned} & \text { Ala } \\ & 1 \end{aligned}$ | Ala Ser | $\begin{aligned} & \text { Ala } \\ & \\ & \hline \end{aligned}$ | $\begin{aligned} & \text { Tyr } \\ & 5 \end{aligned}$ | Ala | Asp | Glu | $\begin{array}{r} \text { Leu } \begin{array}{c} V \\ 1 \end{array} \end{array}$ | $\begin{aligned} & \text { Val I } \\ & 10 \end{aligned}$ | Lys T | Thr | a | Lys | $\begin{aligned} & \text { Thr Ile } \\ & 15 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ala | Ser Pro | $\begin{aligned} & \text { Gly H } \\ & 20 \end{aligned}$ | His | Gly | Ile | Met | Ala M $25$ | Met | Asp | Glu | Ser | $\begin{aligned} & \text { Asn } \\ & 30 \end{aligned}$ | Ala Thr |
| Cys | $\begin{array}{cl} \text { Gly Lys } \\ 35 \end{array}$ | Arg L | Leu | Ala | Ser | $\begin{aligned} & \text { Ile } \\ & 40 \end{aligned}$ | Gly L | Leu. | $\text { Glu } A$ | Asn | $\begin{aligned} & \text { Thr } \\ & 45 \end{aligned}$ |  | Ala Asn |
| Arg | $\begin{aligned} & \text { Gln Ala } \\ & 50 \end{aligned}$ | Tyr | Arg | Thr | $\begin{aligned} & \text { Leu } \\ & 55 \end{aligned}$ | Leu | Val S | Ser | Ala | $\begin{aligned} & \text { Pro } \\ & 60 \end{aligned}$ | Gly | Leu | Gly Gln |
| $\begin{aligned} & \text { Tyr } \\ & 65 \end{aligned}$ | Ile Ser | Gly A | Ala | $\begin{aligned} & \text { Ile } \\ & 70 \end{aligned}$ | Leu | le | $1 \mathrm{u}$ | Glu | $\begin{aligned} & \text { Thr } \\ & 75 \end{aligned}$ | Leu | Tyr | $1 n$ | $\begin{aligned} \text { Ser Thr } \\ 80 \end{aligned}$ |
| Thr | Asp Gly | $\text { Lys } \begin{array}{r} \text { L } \\ 8 \end{array}$ | $\begin{aligned} & \text { Lys } \\ & 85 \end{aligned}$ | Met | Val | Asp | Val | $\begin{aligned} & \text { Leu } \\ & 90 \end{aligned}$ |  | Glu | Gln | Asn | Ile Val 95 |
| Pro | Gly Ile | $\begin{aligned} & \text { Lys V } \\ & 100 \end{aligned}$ | Val | Asp | Lys | Gly | $\begin{aligned} & \text { Leu V } \\ & 105 \end{aligned}$ | Val | ro I | «eu | al | $\begin{aligned} & \text { Gly } \\ & 110 \end{aligned}$ | Ser Tyr |
| Asp | $\begin{aligned} & \text { Glu } \text { Ser } \\ & 115 \end{aligned}$ | $\operatorname{Trp}$ | Cys | Gln | Gly | Leu $120$ | Asp | Gly | eu | la | $\begin{aligned} & \text { Ser A } \\ & 125 \end{aligned}$ | rg | Thr Ala |
| Ala | $\begin{aligned} & \text { Tyr Tyr } \\ & 130 \end{aligned}$ | $\mathrm{Gln}$ | $\mathrm{Gln}$ | Gly | $\begin{aligned} & \text { Ala } \\ & 135 \end{aligned}$ | Arg | Phe A | Ala | $\begin{array}{rr} \text { Lys } & \text { T } \\ 1 \end{array}$ | $\begin{aligned} & \text { Trp } \\ & 140 \end{aligned}$ | Arg | Thr | Val Val |
| $\begin{aligned} & \text { Ser } \\ & 145 \end{aligned}$ | Ile Pro | Asn G | $\mathrm{Gly}$ | $\begin{aligned} & \text { Pro } \\ & 150 \end{aligned}$ | Ser | $1 a$ | eu A |  | $\begin{aligned} & \text { Val } \\ & 155 \end{aligned}$ | Lys | Glu | $1 a$ | $\begin{array}{r} \text { lap } \\ 160 \end{array}$ |
| Gly | Leu Ala | Arg $T$ | $\begin{aligned} & \text { Tyr } \\ & 165 \end{aligned}$ | Ala | Ala | Ile | er $1$ | $\begin{aligned} & \mathrm{Gln} \mathrm{~F} \\ & 170 \end{aligned}$ | Asp S | Ser | Gly | Leu | $\begin{aligned} & \text { Val Pro } \\ & 175 \end{aligned}$ |
| Ile | Val Glu | Pro G $180$ | Glu | Ile | let | eu | $\begin{aligned} & \text { Asp } \\ & 185 \end{aligned}$ | Gly | Glu | His | Gly | $\begin{aligned} & \text { Ile } \\ & 190 \end{aligned}$ | Asp Arg |
| Thr | $\begin{array}{r} \text { Tyr Asp } \\ 195 \end{array}$ | Val A | Ala | Glu | Lys | $\begin{aligned} & \mathrm{Val} \\ & 200 \end{aligned}$ | $\operatorname{Trp} A$ | Ala | Glu | al | Phe $205$ | Phe | Tyr Leu |
| Ala | $\begin{aligned} & \text { Gln Asn } \\ & 210 \end{aligned}$ | Asn | Val | Met | $\begin{aligned} & \text { Phe } \\ & 215 \end{aligned}$ | Glu | Gly | Ile | Leu | $\begin{aligned} & \text { Leu } \\ & 220 \end{aligned}$ | Lys | Pro | Ser Met |
| Val | Thr Pro | Gly A | Ala | Glu | Ala | Thr | Asp A | Arg | Ala T | Thr P | Pro | Glu | Gln Val |


$<210>$ SEQ ID NO 17
$<211>$ LENGTH: 352
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 17


$<210>$ SEQ ID NO 18
$<211>$ LENGTH: 353
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Hordeum Vulgare
$<400>$ SEQUENCE: 18


|  | 290 |  |  |  |  | 295 |  |  |  |  | 300 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \text { Gln } \\ & 305 \end{aligned}$ | Gly | Gln | Pro | $\mathrm{Glu}$ | $\begin{aligned} & \text { Asn } \\ & 310 \end{aligned}$ | Ile | Glu | Ala | Ala | $\begin{aligned} & \text { Gln } \\ & 315 \end{aligned}$ | Lys Ala | Leu | $\begin{array}{r} \text { Leu Val } \\ 320 \end{array}$ |
| Arg | Ala | Lys | Ala | $\begin{aligned} & \text { Asn } \\ & 325 \end{aligned}$ | Ser | Leu | Ala | Gln | $\begin{aligned} & \text { Leu } \\ & 330 \end{aligned}$ | Gly | Ser Tyr | Thr | $\begin{aligned} & \text { Gly Glu } \\ & 335 \end{aligned}$ |
| Gly | Glu | Ser | $\begin{aligned} & \text { Asp } \\ & 340 \end{aligned}$ | Glu | Ala | Lys | Lys | $\begin{aligned} & \text { Gly } \\ & 345 \end{aligned}$ | Met | Phe | Gln Lys | $\begin{aligned} & \text { Gly } \\ & 350 \end{aligned}$ | Tyr Thr |
| Tyr |  |  |  |  |  |  |  |  |  |  |  |  |  |

$<210>$ SEQ ID NO 19
$<211>$ LENGTH: 244
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Hordeum vulgare
$<400>$ SEQUENCE: 19

$<210>$ SEQ ID NO 20
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Hordeum vulgare

$<400>$ SEQUENCE $: 20$


$<210>$ SEQ ID NO 21
$<211>$ LENGTH: 352
$<212>$ TYPE : PRT
$<213>$ ORGANISM: Lycopersicon esculentum
$<400>$ SEQUENCE: 21



$<210>$ SEQ ID NO 23
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Lycopersicon esculentum
$<400>$ SEQUENCE: 23


$<210>$ SEQ ID NO 24
$<211>$ LENGTH: 339
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Lotus japonicus
$<400>$ SEQUENCE: 24


$<210>$ SEQ ID NO 25
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Lotus japonicus
$<400>$ SEQUENCE: 25


$<210>$ SEQ ID NO 26
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Lotus japonicus
$<400>$ SEQUENCE : 26


$<210>$ SEQ ID NO 27
$<211>$ LENGTH: 353
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Oryza sativa
$<400>$ SEQUENCE : 27



$<210>$ SEQ ID NO 29
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Picea sitchensis
$<400>$ SEQUENCE: 29


$<210>$ SEQ ID NO 30
$<211>$ LENGTH: 351
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Picea sitchensis
$<400>$ SEQUENCE: 30




```
<210> SEQ ID NO 33
<211> LENGTH: 351
<212> TYPE: PRT
<213> ORGANISM: Populus trichocarpa
<400> SEQUENCE: 33
```

Thr Gly Ser Tyr Ala Glu Glu Leu Val Lys Thr Ala Lys Thr Ile Ala

$<210>$ SEQ ID NO 34
$<211>$ LENGTH: 245
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Zea mays
$<400>$ SEQUENCE: 34


$<210>$ SEQ ID NO 35
$<211>$ LENGTH: 350
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Glycine max
$<400>$ SEQUENCE: 35


$<210>$ SEQ ID NO 36
$<211>$ LENGTH: 358
$<212>$ TYPE: PRT
$<213>$ ORGANISM: Arabidopsis thaliana
$<400>$ SEQUENCE: 36

 6SOT ：HLSNHT＜TIて＞ $8 \varepsilon$ ON GI ÖHS＜OLZ＞


| 0 T 0．67eb6ebo |  |
| :---: | :---: |
|  |  |
















euetteyl stsdoptqexv ：WSINED甘O＜عTZ＞
ZND ：HdAL＜ZIと〉
9SOT ：HLDNGT＜TTZ＞ Lع ON GI Õت̃S＜OIZ＞

Sรを

0รを Gモ\＆0も



OZE STE OTE SOE
ety utp ety sit ety ten usy nip oxd sit
$00 \varepsilon \quad 96 Z 06 Z$

与8Z 08Z SLZ
 $0 \angle Z$ G9Z 09Z
 ŞZ OSZ Sもて



0ZZ STZ 0TZ

$\qquad$
pənut 子uoŋ－











 7666670067 จ6ee66eep 67067706e6 707e0．e660 ev70077e06 e07677670e








әле6โn＾unәрлон ：WSIN甘ゆ\＆o＜とโて＞
चNG ：HCXL＜ZTZ〉
乙とL ：HLDN＇TT＜TLて＞


е6 70e70．еред จ666eebedo 7767eo666e eberoo66e6


















əле6โn＾unəpxoH ：WSINZDYO＜\＆T乙＞
ZND ：GdXL＜ZTZ＞ z90T：HWDN甘T＜TIZ＞ Oも ON AI ÕGS＜OTZ＞





əлย6TnA unepaOH ：WSINEゆYO＜とTZ＞
ZND ：GオXL＜ZIZ＞
9SOT ：HWDNGT＜ITZ＞



 $09 \varepsilon$ де6770766e eจっ6766770 qee67eepee eっ77667066 77700776e7 77666eepe6




 Sも：GDNHOÕES＜OOも＞

ZNG ：出dXL＜ZTZ＞ 9SOT ：HWゆNGT＜T．Z〉 Sも ON GI Õas＜OTZ＞


|  |  |  |
| :---: | :---: | :---: |
|  |  |  |











 7766ее77е7 667ро77677 ерее6ер6е6 77677р6767 е677667еее е6еее666е6






 990T ：HLDNAT＜TTZ＞
























9SOL HLDN＇AT＜TLて
8 も ON GI ÖJS＜OTZ＞



$$
6 も \text { : GONGกÕتُ }
$$

enţes ezKio ：NSINEDYO＜\＆Z＞
ENG ：GCKL＜ZIて＞ Z90T ：HW9NGT＜TTZ＞ 6も ON GI ÕتtS＜OTZ＞

－WSINZゆ\＆o＜とIて＞

品











 9SOT ：HLDN＇HT＜TLて＞ TS ON GI Ö푸 \llOIZ＞



















OS ：GDNHOO゙GS＜OOも＞
EATZes ezKiO ：WSTNED\＆O＜\＆TZ＞
ZNG：島dXL＜てTと＞
9SOT ：HWDNGT＜TTZ＞ OG ON GI Õ＇ت゙S＜OLZ＞













$00 \varepsilon$





モG：GDNA

चNG ：马ुオX山＜ZTZ＞ 990T ：HLDNGT＜TIZ＞

















edxepoчptxq sntndod ：WSINEDYO＜とT乙＞
ZND ：JdXL＜ZTZ＞
 $\varepsilon \subseteq$ ON aI Õ＇ت̉S＜OIZ＞




| gaggtggagg cgactctgaa cetgaatgcg atgaaccagt ctccgaacce atggcacgta | 540 |
| :--- | :---: |
| tcattctcct acgcccgggc tctgcagaac tcggtgctga agacatggca agggcgcccc | 600 |
| gagaacgttg aggcggcgca aaggccetg ctggtgcgcg caaaggccaa ctcgctggca | 660 |
| cagctaggtc gctacactgg tgagggtgag agcgacgagg cgaagaaagg catgttccag | 720 |
| aagggctaca cotactaa | 738 |

The invention claimed is:

1. A method for producing a plant with increased sugar content relative to a corresponding untreated plant, the method comprising the steps of:
applying glutathione to a plant or soil in which a plant is to be cultivated;
cultivating the plant under conditions suitable for growth; and
selecting for a plant having increased sugar content relative to a corresponding untreated plant.
2. The method according to claim 1, wherein the glutathione is oxidized glutathione.
3. The method according to claim 1, wherein the step of 2.5 applying the effective amount of glutathione comprises applying 0.01 mM to 20 mM to the plant or soil.
4. The method according to claim 1, wherein the step of applying the effective amount of glutathione comprises applying 0.1 mM to 2 mM to the plant or soil.
5. The method according to claim 1 , wherein the step of applying the effective amount of glutathione to the plant comprises applying glutathione to an entire plant, or to one or more portions of the plant.
6. The method according to claim $\mathbf{1}$, wherein the plant is grown from one or more seeds, and the step of applying the effective amount of glutathione comprises applying in regular intervals for at least 30 days beginning on the day of sowing the seeds.
7. The method according to claim 6 , wherein the step of applying the effective amount of glutathione comprises applying in regular intervals for at least 60 days beginning on 15 the day of sowing.
8. The method according to claim 6, further comprising harvesting the plant, and wherein the step of applying the effective amount of glutathione comprises applying glutathione one to four times per week from the day of sowing until harvesting.
9. The method according to claim 8 , wherein glutathione is applied one to four times per week in an amount of 0.001-0.1 mmol per application.
10. The method of claim 1 , wherein the step of applying the effective amount of glutathione comprises applying glutathione in regular intervals selected from the group consisting of: until bud break, after flower petals have fallen, from bud break until production of fruit, from flowering time until production of fruit, or from a time after petals have fallen until 0 production of fruit.
11. The method of claim 1 , further comprising harvesting the plant, and wherein the step of applying the effective amount of glutathione comprises applying glutathione in regular intervals 10 days before harvesting until harvesting.
12. The method of claim 1 , further comprising harvesting the plant, and wherein the step of applying the effective amount of glutathione comprises applying glutathione in regular intervals 20 days before harvesting until harvesting.
