United States Patent	12385019
Kind Code	B2
Date of Patent	August 12, 2025
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Buckwheat-derived C-glycosyltransferase gene and utilization thereof

Abstract

Transgenic plants with blue flower color, or their inbred or outbred progeny, or their propagules, partial plant bodies, tissues or cells, are provided. A buckwheat-derived C-glucosyltransferase (CGT) gene or its homolog is transferred into a host plant to cause delphinidin-type anthocyanins and flavone mono-C-glycosides to be copresent in the plant cells.

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Appl. No.: **17/765692**

Filed (or PCT Filed): **September 25, 2020**

PCT No.: **PCT/JP2020/036451**

PCT Pub. No.: **WO2021/065749**

PCT Pub. Date: April 08, 2021

Prior Publication Data

Document Identifier	Publication Date
US 20230220357 A1	Jul. 13, 2023

Foreign Application Priority Data

JP	2019-181693	Oct. 01, 2019

Publication Classification

Int. Cl.: **A01H5/02** (20180101); **A01H6/74** (20180101); **C12N9/02** (20060101); **C12N9/10** (20060101); **C12N9/88** (20060101); **C12N15/82** (20060101)

U.S. Cl.:

CPC **C12N9/1051** (20130101); **A01H5/02** (20130101); **A01H6/749** (20180501); **C12N9/0071** (20130101); **C12N9/1007** (20130101); **C12N9/88** (20130101); **C12N15/825** (20130101); **C12Y114/14** (20130101); **C12Y201/01** (20130101); **C12Y204/01** (20130101); **C12Y402/01105** (20130101);

Field of Classification Search

USPC: None

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2020/0283782	12/2019	Nakamura et al.	N/A	N/A

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Background/Summary

FIELD

(1) The present invention relates to buckwheat-derived C-glucosyltransferase (CGT) genes or their homologs, and to a method for creating transgenic plants with blue flower color, comprising a step of using the genes to cause delphinidin-type anthocyanins and flavone mono-C-glycosides to be copresent in plant cells.

BACKGROUND

(2) Rose, chrysanthemum and carnation are industrially important ornamental flowers worldwide. Rose in particular, being the most popular flowering plant, has a record of cultivation since ancient times, and it has been artificially crossbred for hundreds of years. One problem, however, has been that none of the hybridizable related species have wild varieties with blue flower color, and it has therefore been difficult to create rose varieties with blue flower color by conventional cross-breeding and mutation breeding. Creating completely new blue flower colors should lead to new demand for even wider uses of ornamental flowers, and should help to increase production and consumption. It has therefore been attempted to create roses with blue flower colors by genetic engineering methods.

(3) Flowers with purple to blue colors, for example, are known to abundantly contain delphinidin-type anthocyanins having delphinidin, petunidin and malvidin backbones, but since ornamental flowers such as rose cannot produce such delphinidin-type anthocyanins, research continues to be conducted with the aim of artificially producing delphinidins by expressing the flavonoid 3′,5′-hydroxylase gene that is necessary for their synthesis (NPL 1). However, even when plant metabolism is artificially modified in order to express an enzyme gene that produces a substance of interest in the recombinant plant, often little or absolutely none of the substance of interest accumulates.

(4) Moreover, the color of a flower changes not only by the structures of the anthocyanins themselves as the essential pigments, but also due to copresent flavonoids (also known as copigments), metal ions, and the vacuole pH. Flavones or flavonols are typical copigments that form sandwich-like layers with anthocyanins and render the anthocyanins blue, producing a deepening effect (NPL 2). This is known as the "copigment effect". Flavones, in particular, are known to exhibit a powerful copigment effect, and analysis of gene recombinant carnations, for

example, has demonstrated that flavones exhibit a significant copigment effect (NPL 3). For Dutch iris, it has been reported that a higher ratio of the total flavone content with respect to the total delphinidin content results in a more powerful copigment effect, and a bluer color (NPL 4).
(5) However, not all plants can produce flavones, and it is known that roses and petunias do not store flavones. Attempts have therefore been made to modify flower color by expressing in the plants different genes coding for proteins having activity for synthesizing flavones from flavanones (PTL 1).
(6) In plants, flavones are distributed not only in free form but also as glycosides, with flavone O-glycosides and flavone C-glycosides being formed primarily, and flavone C-glycosides being known to exhibit a particularly powerful copigment effect. For example, isovitexin, as one type of flavone C-glycoside, has been reported to exhibit a copigment effect with anthocyanins in Japanese garden iris (*Iris ensata* Thunb.), and to produce a blue flower color via stabilization of anthocyanins (NPL 5). One known biosynthetic pathway for flavone C-glycosides is synthesis from flavanones via reaction catalyzed by flavanone 2-hydroxylase (F2H), C-glucosyltransferase (CGT) and dehydratase (FDH) (NPL 6).
(7) Previously it has been reported that roses with blue flower color were created by introducing the *Campanula*-derived F3′,5′H gene and the torenia-derived MT gene, licorice-derived F2H gene, rice-derived CGT gene and *Lotus japonicus*-derived FDH gene, to cause copresence of delphinidin-type anthocyanins and flavone C-glycosides in plant cells (PTL 2). However, the flower colors of rose varieties created in this manner are found to have a strong reddish tint, and it is therefore still desired to develop techniques for controlling blue color expression in order to allow uniform and stable creation of roses with bluer flower colors.

CITATION LIST

Patent Literature

(8) [PTL 1] Japanese Unexamined Patent Publication No. 2000-279182 [PTL 2] International Patent Publication No. 2019/069946 [PTL 3] International Patent Publication No. 2008/156206

Non Patent Literature

(9) [NPL 1] Phytochemistry Reviews 5, 283-291 [NPL 2] Prog. Chem. Org. Natl. Prod. 52 [NPL 3] Phytochemistry, 63, 15-23(2003) [NPL 4] Plant Physiol. Bioch. 72, 116-124(2013) [NPL 5] Euphytica 115, 1-5(2000) [NPL 6] FEBS Lett. 589, 182-187(2015)

SUMMARY

Technical Problem

(10) The problem to be solved by the invention is to create a transgenic plant having a uniform and stable blue flower color (RHS Color Chart 5th Edition: Violet-Blue group/Blue group and/or hue angle: 339.7°-270.0°), based on research on the causes of redness in flower color.

Solution to Problem

(11) As a result of ardent research and much experimentation on this problem, the present inventors have found that flavone di-C-glycosides are a cause of redness, and that the copigment effect of flavone mono-C-glycosides with delphinidin-type anthocyanins is significantly higher than that of flavone di-C-glycosides. By transferring the buckwheat-derived CGT gene, which was selected from among various CGT genes, the present inventors further succeeded in significantly accumulating only flavone mono-C-glycosides in plant petals. The invention has been completed upon these findings.
(12) Specifically, the present invention provides the following.
(13) [1] A buckwheat-derived CGT gene or its homolog, wherein:
(14) the buckwheat-derived CGT gene or its homolog is selected from the group consisting of:
(15) (1-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 11;
(16) (1-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 11, under stringent conditions, and has the same activity as the polynucleotide of (1-a); and

(17) (1-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 12.
(18) [2] The buckwheat-derived CGT gene or its homolog according to [1], to which the *Arabidopsis thaliana* ADH gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or the *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) has been added.
(19) [3] A vector comprising a buckwheat-derived CGT gene or its homolog, wherein the buckwheat-derived CGT gene or its homolog is selected from the group consisting of:
(20) (1-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 11;
(21) (1-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 11 under stringent conditions and has the same activity as the polynucleotide of (1-a);
(22) (1-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 12;
(23) (1-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 12 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (1-c); and
(24) (1-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 12 and having the same activity as a protein encoded by the polynucleotide of (1-c).
(25) [4] The vector according to [3], wherein the *Arabidopsis thaliana* ADH gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or the *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) has been added to the buckwheat-derived CGT gene or its homolog.
(26) [5] The vector according to [3] or [4], which further comprises a flavanone 2-hydroxylase (F2H) gene or its homolog, and a dehydratase (FDH) gene or its homolog.
(27) [6] The vector according to [5], wherein
(28) the F2H gene or its homolog is selected from the group consisting of:
(29) (2-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 5;
(30) (2-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 5 under stringent conditions and has the same activity as the polynucleotide of (2-a);
(31) (2-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 6;
(32) (2-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 6 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (2-c); and
(33) (2-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 6 and having the same activity as a protein encoded by the polynucleotide of (2-c), and
(34) the FDH gene or its homolog is selected from the group consisting of:
(35) (3-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 9;
(36) (3-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 9 under stringent conditions and has the same activity as the polynucleotide of (3-a);
(37) (3-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 10;
(38) (3-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the

amino acid sequence listed as SEQ ID NO: 10 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (3-c); and
(39) (3-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 10 and having the same activity as a protein encoded by the polynucleotide of (3-c).
(40) [7] The vector according to any one of [3] to [6], which further includes a flavonoid F3′,5′ hydroxylase (F3′,5′H) gene or its homolog, and a methyltransferase (MT) gene or its homolog.
(41) [8] The vector according to [7], wherein
(42) the F3′,5′H gene or its homolog is selected from the group consisting of:
(43) (4-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 1;
(44) (4-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 1 under stringent conditions and has the same activity as the polynucleotide of (4-a);
(45) (4-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 2;
(46) (4-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 2 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (4-c); and
(47) (4-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 2 and having the same activity as a protein encoded by the polynucleotide of (4-c), and
(48) the MT gene or its homolog is selected from the group consisting of:
(49) (5-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 3;
(50) (5-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 3 under stringent conditions and has the same activity as the polynucleotide of (5-a);
(51) (5-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 4;
(52) (5-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 4 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (5-c); and
(53) (5-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 4 and having the same activity as a protein encoded by the polynucleotide of (5-c).
(54) [9] A transgenic plant comprising a buckwheat-derived CGT gene or its homolog according to [1] or [2] or a vector according to any one of [3] to [8], or its inbred or outbred progeny.
(55) [10] The transgenic plant according to [9], or its inbred or outbred progeny, wherein the plant is selected from among rose, chrysanthemum, carnation or lily.
(56) [11] The transgenic plant according to [10], or its inbred or outbred progeny, wherein the plant is rose.
(57) [12] Propagules, partial plant bodies, tissue or cells of a transgenic plant according to any one of [9] to [11] or its inbred or outbred progeny.
(58) [13] Cut flowers of a transgenic plant according to any one of [9] to [11], or its inbred or outbred progeny, or a processed form created from the cut flowers.
(59) [14] A method for creating transgenic plants with blue flower color, comprising a step of transferring a buckwheat-derived C-glucosyltransferase (CGT) gene or its homolog into a host plant to cause delphinidin-type anthocyanins and flavone mono-C-glycosides to coexist in the plant

cells.
(60) [15] The method according to [14], wherein the flavone mono-C-glycoside is apigenin 6-C-glucoside, luteolin 6-C-glucoside, tricetin 6-C-glucoside, apigenin 8-C-glucoside, luteolin 8-C-glucoside or tricetin 8-C-glucoside, or a derivative thereof.
(61) [16] The method according to [14] or [15], wherein the delphinidin-type anthocyanin is selected from the group consisting of malvidins, delphinidins, petunidins and their combinations.
(62) [17] The method according to any one of [14] to [16], which further comprises transferring a buckwheat-derived CGT gene or its homolog according to [1] or [2] or a vector according to any one of [3] to [8] into host plant cells.
(63) [18] The method according to any one of [14] to [17], wherein the plant is rose, chrysanthemum, carnation or lily.
(64) [19] The method according to [18], wherein the plant is rose.
Advantageous Effects of Invention
(65) According to the invention it is possible to uniformly and stably create a plant variety having a blue flower color (RHS Color Chart 5th Edition: Violet-Blue group/Blue group and/or hue angle: 339.7°-270.0°).

Description

BRIEF DESCRIPTION OF DRAWINGS
(1) FIG. **1** shows the biosynthetic pathway for a flavone mono-C-glycoside in a plant.
(2) FIG. **2** shows the structure of pSPB6486.
(3) FIG. **3** shows the structure of pSPB7473.
(4) FIG. **4** shows the detailed structured of pSPB7473.
(5) FIG. **5** shows the detailed structured of pSPB7472.
(6) FIG. **6** shows the detailed structured of pSPB7808.
(7) FIG. **7** shows the detailed structured of pSPB7809.
(8) Anthocyanins are a group of pigments that are widely extant in plants, and they are known to exhibit red, blue and purple flower colors. They are classified into 3 types, pelargonidin, cyanidin and delphinidin, based on the number of hydroxyl groups on the B-ring of the anthocyanidin, as the aglycone form. The chromophoric group is the aglycone portion, with pelargonidin-type anthocyanins exhibiting orange color, cyanidin-type anthocyanins exhibiting red color and delphinidin-type anthocyanins exhibiting purple to blue color. Throughout the present specification, “delphinidin-type anthocyanins” also include their derivatives having delphinidin, malvidin or petunidin backbones, with malvidin being preferred.
(9) When delphinidin-type anthocyanins are copresent with substances such as flavones, flavonols, organic acid esters and tannins, their molecular interaction often produces blueish colors. This phenomenon is known as “copigmentation”, and substances that produce the phenomenon are known as copigments. Copigmentation includes not only a color depth effect that induces blue color production, but also a deep color effect or an effect of increasing color stability. The present inventors have confirmed that copigmentation between delphinidin-type anthocyanins and flavone C-glycosides causes blue color expression in rose petals (PTL 2).
(10) Flavones are organic compounds that are flavan-derived cyclic ketones, and in plants they mainly exist as glycosides. Flavone, in the strict definition, refers to 2,3-didehydroflavan-4-one, which is a compound with chemical formula C.sub.15H.sub.10O.sub.2 and molecular weight 222.24, but in the wider sense flavones are a category of flavonoids, a flavonoid being classified as a “flavone” if it has a flavone structure as the basic backbone and also lacks the hydroxyl group at the 3-position. As used herein, “flavone C-glycoside” means a glycoside of a flavone in the wide sense, i.e. a derivative falling under the definition of flavones, wherein an aglycone is directly

bonded to the anomeric carbon of an aldose. Flavone C-glycosides include, but are not limited to, luteolin C-glycoside, tricetin C-glycoside, apigenin C-glycoside and acacetin C-glycoside.
(11) Flavone C-glycosides also include glycosides of apigenin, luteolin, tricetin and acacetin derivatives. One pathway known for the biosynthetic pathway of flavone C-glycosides in plants is the pathway shown in FIG. **1**. In this pathway, flavone C-glycoside is produced via F2H, CGT and FDH.
(12) In this synthesis pathway, flavone di-C-glycosides such as vicenin-2 (apigenin 6,8-di-C-glucoside) are also synthesized in addition to flavone mono-C-glycosides, but the present inventors have found, surprisingly, that flavone di-C-glycosides are a cause of redness, and that flavone mono-C-glycosides have a higher copigment effect with delphinidin-type anthocyanins than flavone di-C-glycosides. In order to uniformly and stably create a transgenic plant with blue flower color, it is necessary to reduce to a minimum the accumulation of flavone di-C-glycosides, and to accumulate significant amounts of flavone mono-C-glycosides alone in the petals. Flavone mono-C-glycosides are typically flavone 6-C-glucosides or flavone 8-C-glucosides, but are preferably flavone 6-C-glucosides. Examples of flavone mono-C-glycosides include apigenin 6-C-glucoside (isovitexin), luteolin 6-C-glucoside (isoorientin), tricetin 6-C-glucoside, apigenin 8-C-glucoside (vitexin), luteolin 8-C-glucoside (orientin) and tricetin 8-C-glucoside, or their derivatives.
(13) Accumulation of flavone C-glycosides in plant cells can be achieved by transforming host plants with vectors comprising genes necessary for the aforementioned synthesis pathways (i.e. the F2H gene, CGT gene and FDH gene), or their homologs. If the CGT gene used is a buckwheat-derived CGT gene or its homolog, and particularly a buckwheat-derived CGT gene or its homolog with the *Arabidopsis thaliana* ADH gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) added as a translation enhancing sequence, it is possible to accumulate significantly more flavone mono-C-glycosides in petals than flavone di-C-glycosides.
(14) The buckwheat-derived CGT gene or its homolog is selected from the group consisting of the following polynucleotides:
(15) (1-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 11;
(16) (1-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 11 under stringent conditions and has the same activity as the polynucleotide of (1-a);
(17) (1-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 12;
(18) (1-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 12 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (1-c); and
(19) (1-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 12 and having the same activity as a protein encoded by the polynucleotide of (1-c).
(20) The source of the F2H gene or its homolog is not particularly restricted so long as it has the desired function, but it is preferably a licorice-derived F2H gene or its homolog, and selected from the group consisting of the following polynucleotides:
(21) (2-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 5;
(22) (2-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 5 under stringent conditions and has the same activity as the polynucleotide of (2-a);
(23) (2-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 6;
(24) (2-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the

amino acid sequence listed as SEQ ID NO: 6 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (2-c); and
(25) (2-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 6 and having the same activity as a protein encoded by the polynucleotide of (2-c).
(26) The source of the FDH gene or its homolog is not particularly restricted so long as it has the desired function, but it is preferably a *Lotus japonicus*-derived FDH gene or its homolog, and selected from the group consisting of the following polynucleotides:
(27) (3-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 9;
(28) (3-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 9 under stringent conditions and has the same activity as the polynucleotide of (3-a);
(29) (3-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 10;
(30) (3-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 10 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (3-c); and
(31) (3-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 10 and having the same activity as a protein encoded by the polynucleotide of (3-c).
(32) Accumulation of delphinidin-type anthocyanins in plant cells can be achieved by incorporating a flavonoid F3′,5′ hydroxylase (F3′,5′H) gene or its homolog and a methyltransferase (MT) gene or its homolog in a host plant (PTL 3). By transforming a host plant with a vector further comprising a F3′,5′H gene or its homolog and an MT gene or its homolog in addition to a gene necessary for the synthesis pathway of the aforementioned flavone mono-C-glycosides or their homologs, it is possible to cause a delphinidin-type anthocyanin and a flavone mono-C-glycoside to coexist in the host plant cells.
(33) The source of the F3′,5′H gene or its homolog is not particularly restricted so long as it has the desired function, but it is preferably a *Campanula*-derived F3′,5′H gene or its homolog, and selected from the group consisting of:
(34) (4-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 1;
(35) (4-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide sequence complementary to the nucleotide sequence listed as SEQ ID NO: 1 under stringent conditions and has the same activity as the polynucleotide of (4-a);
(36) (4-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 2;
(37) (4-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 2 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (4-c); and
(38) (4-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 2 and having the same activity as a protein encoded by the polynucleotide of (4-c).
(39) The source of the MT gene or its homolog is not particularly restricted so long as it has the desired function, but it is preferably a torenia-derived MT gene or its homolog, and selected from the group consisting of:
(40) (5-a) a polynucleotide consisting of the nucleotide sequence listed as SEQ ID NO: 3;
(41) (5-b) a polynucleotide that hybridizes with a polynucleotide consisting of the nucleotide

sequence complementary to the nucleotide sequence listed as SEQ ID NO: 3 under stringent conditions and has the same activity as the polynucleotide of (5-a);
(42) (5-c) a polynucleotide that encodes a protein consisting of the amino acid sequence listed as SEQ ID NO: 4;
(43) (5-d) a polynucleotide that encodes a protein consisting of an amino acid sequence that is the amino acid sequence listed as SEQ ID NO: 4 having a deletion, substitution, insertion and/or addition of one or more amino acids, and having the same activity as a protein encoded by the polynucleotide of (5-c); and
(44) (5-e) a polynucleotide that encodes a protein having an amino acid sequence with at least 90% identity with respect to the amino acid sequence listed as SEQ ID NO: 4 and having the same activity as a protein encoded by the polynucleotide of (5-c).
(45) The *Arabidopsis thaliana* ADH gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or the *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) may also be added as a translation enhancing sequence to the MT gene or its homolog.
(46) Throughout the present specification, the term “polynucleotide” refers to DNA or RNA.
(47) As used herein, the term “stringent conditions” refers to conditions that allow specific binding between a polynucleotide or oligonucleotide and genomic DNA in a selective and detectable manner. Stringent conditions are defined by an appropriate combination of salt concentration, organic solvent (for example, formamide), temperature and other known conditions. Specifically, stringency is increased by reducing the salt concentration, increasing the organic solvent concentration or raising the hybridization temperature. Stringency is also affected by the rinsing conditions after hybridization. The rinsing conditions are defined by the salt concentration and temperature, and stringency of rinsing is increased by reducing the salt concentration and raising the temperature. Therefore, the term “stringent conditions” means conditions such that specific hybridization takes place only between nucleotide sequences with high identity, such as a degree of “identity” between the nucleotide sequences of about 80% or greater, preferably about 90% or greater, more preferably about 95% or greater, even more preferably 97% or greater and most preferably 98% or greater, on average. The “stringent conditions” may be, for example, a temperature of 60° C. to 68° C., a sodium concentration of 150 to 900 mM and preferably 600 to 900 mM, and a pH of 6 to 8, with specific examples including hybridization under conditions of 5×SSC (750 mM NaCl, 75 mM trisodium citrate), 1% SDS, 5×Denhardt solution, 50% formaldehyde, 42° C., and rinsing under conditions of 0.1×SSC (15 mM NaCl, 1.5 mM trisodium citrate), 0.1% SDS, 55° C.
(48) The hybridization may be carried out by a method that is publicly known in the field or a similar method, such as the method described in Current Protocols in Molecular Biology (edited by Frederick M. Ausubel et al., 1987). When a commercially available library is to be used, the hybridization may be carried out according to the method described in the accompanying directions for use. The gene selected by hybridization may be naturally derived, such as plant-derived or non-plant-derived. The gene selected by the hybridization may be cDNA, genomic DNA or chemically synthesized DNA.
(49) The phrase “amino acid sequence having a deletion, substitution, insertion and/or addition of one or more amino acids”, as used herein, means an amino acid sequence having a deletion, substitution, insertion and/or addition of 1 to 20, preferably 1 to 5 and more preferably 1 to 3 arbitrary amino acids. Site-specific mutagenesis is a useful genetic engineering method as it allows introduction of specific mutations into specified sites, and it may be carried out by the method described in Molecular Cloning: A Laboratory Manual, 2.sup.nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. By expressing the mutant DNA using a suitable expression system, it is possible to obtain a protein consisting of an amino acid sequence having a deletion, substitution, insertion and/or addition of one or more amino acids.
(50) A polynucleotide can be obtained by a method that is publicly known to those skilled in the

art, such as a method of chemical synthesis using the phosphoramidite method, or a nucleic acid amplification method using a plant nucleic acid specimen as template, and primers designed based on the nucleotide sequence of the target gene.

(51) Throughout the present specification, the term “identity” means, for polypeptide sequences (or amino acid sequences) or polynucleotide sequences (or nucleotide sequences), the quantity (number) of amino acid residues or nucleotides composing them that can be determined to be identical between the two chains, in the sense of mutual agreement between them, meaning the degree of sequence correlation between two polypeptide sequences or two polynucleotide sequences, and this “identity” can be easily calculated. Numerous methods are known for measuring identity between two polynucleotide sequences or polypeptide sequences, and the term “identity” is well known to those skilled in the art (for example, see Lesk, A. M. (Ed.), Computational Molecular Biology, Oxford University Press, New York, (1988); Smith, D. W. (Ed.), Biocomputing: Informatics and Genome Projects, Academic Press, New York, (1993); Grifin, A. M. & Grifin, H. G. (Ed.), Computer Analysis of Sequence Data: Part I, Human Press, New Jersey, (1994); von Heinje, G., Sequence Analysis in Molecular Biology, Academic Press, New York, (1987); Gribskov, M. & Devereux, J. (Ed.), Sequence Analysis Primer, M-Stockton Press, New York, (1991) and elsewhere).

(52) Also, the numerical values for “identity” used in the present specification, unless otherwise specified, may be the numerical values calculated using an identity search program known to those skilled in the art, but they are preferably numerical values calculated using the ClustalW program of MacVector Application (version 9.5, Oxford Molecular Ltd., Oxford, England). According to the invention, the degree of “identity” between polynucleotide sequences or amino acid sequences is, for example, about 90% or greater, preferably about 95% or greater, more preferably about 97% or greater, and most preferably about 98% or greater.

(53) The polynucleotide (nucleic acid, gene) of the invention “encodes” a protein of interest. Here, “encodes” means that it allows expression of the protein of interest in a state in which it exhibits its activity. Also, the term “encodes” includes both encoding a structural sequence (exon) that is a continuous section of the protein of interest, and encoding via an intervening sequence (intron).

(54) A gene with a natural nucleotide sequence can be obtained by analysis using a DNA sequencer, for example. Also, DNA encoding an enzyme having a modified amino acid sequence can be synthesized using common site-specific mutagenesis or PCR, based on DNA having the natural nucleotide sequence. For example, a DNA fragment to be modified may be obtained by restriction enzyme treatment of natural cDNA or genomic DNA, and used as template for site-specific mutagenesis or PCR using primers with the desired mutation, to obtain a DNA fragment having the desired modification. The DNA fragment having the mutation may then be linked with a DNA fragment encoding another portion of the target enzyme.

(55) Alternatively, in order to obtain DNA encoding an enzyme consisting of a shortened amino acid sequence, DNA encoding an amino acid sequence longer than the target amino acid sequence, such as the full-length amino acid sequence, may be cut with a desired restriction enzyme, and if the obtained DNA fragment does not code for the full target amino acid sequence, then a DNA fragment consisting of the sequence of the missing portion may be synthesized and linked to it.

(56) By expressing the obtained polynucleotide using a gene expression system in *Escherichia coli* or yeast and measuring the enzyme activity, it is possible to confirm that the obtained polynucleotide encodes a protein with the desired activity.

(57) The present invention relates to a (recombinant) vector, and especially an expression vector, including the aforementioned polynucleotide, and to chrysanthemum plants transformed by the vector.

(58) The vector of the invention also comprises an expression control region, such as a promoter, terminator and replication origin, that are dependent on the type of host plant into which it is introduced. Examples of promoters that constitutively express polynucleotides in plant cells

include cauliflower mosaic virus 35S promoter, El.sub.235S promoter having two 35S promoter enhancer regions linked together, and the rd29A gene promoter, rbcS promoter and mac-1 promoter. For tissue-specific gene expression, a promoter for a gene expressed specifically in that tissue may be used.

(59) The vector may be created by a common method using a restriction enzyme and ligase. Transformation of a host plant using the expression vector may also be carried out by a common method.

(60) At the current level of technology, it is possible to use techniques to introduce a polynucleotide into a plant and constitutively or tissue-specifically express the polynucleotide. Transfer of the DNA into the plant may be carried out by a method known to those skilled in the art, such as the *Agrobacterium* method, binary vector method, electroporation method, PEG method or particle gun method.

(61) Plants to be used as hosts for the invention are not particularly restricted and may be plants belonging to genus Rosaceae *Rosa*, Compositae *Chrysanthemum*, Caryophyllaceae *Dianthus* (such as carnation) or Liliaceae *Lilium*, among which rose cultivar of Rosaceae Rosa (scientific name: *Rosa hybrida*) is especially preferred. The term "rose plant", as used herein, is a rose cultivar of Rosaceae Rosa (scientific name: *Rosa hybrida*), which is its taxonomical classification. Roses are largely classified as Hybrid Tea, *Floribunda* and *Polyantha* roses based on their tree form and flower size, with the major pigment (anthocyanin) in the petals of all lines being of two types, the cyanidin-type and pelargonidin-type. The type of rose plant used as a host for the invention is not particularly restricted, and any of these varieties or lines are suitable. Examples of rose varieties to be used as hosts include Ocean Song, Noblesse, Rita Perfumera, Cool Water, Fame, Topless and Peach Avalanche.

(62) By means of the present invention it is possible to uniformly and stably create transgenic plants, preferably Rosaceae Rosa, Compositae *Chrysanthemum* and Caryophyllaceae *Dianthus* (carnation), and most preferably rose plants, having blue flower colors. When the obtained transgenic plant is a rose plant, it exhibits a flower color in the Blue group or Violet-Blue group according to the RHS Color Chart, and/or with a hue angle of 339.7° to 270.0°, and more preferably 315° or smaller, in the CIEL*a*b* color system.

(63) The invention still further relates to cut flowers of the obtained transgenic plant or its inbred or outbred progeny, or the propagules, partial plant body, tissue or cells, or a processed form created from the cut flowers (especially processed cut flowers). The processed cut flowers referred to here include pressed flowers formed using cut flowers, or preserved flowers, dry flowers or resin sealed products, with no limitation to these.

(64) The present invention will now be explained in greater detail by examples.

EXAMPLES

Example 1: Simulation of Flavone C-Glycoside Copigment Effect with Anthocyanin (Malvin)

(65) An anthocyanin (malvin) and flavone C-glycoside were prepared to simulate the copigment effect of the flavone C-glycoside on malvin. The malvin (malvidin 3,5-diglucoside) and flavone C-glycosides (vitexin (apigenin 8-C-glucoside), isovitexin (apigenin 6-C-glucoside), orientin (luteolin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside)) used in the experiments were purchased from Nacalai Tesque, Inc.

(66) Each flavone C-glycoside (vitexin, isovitexin, orientin, isoorientin or vicenin-2) was added to the acquired malvin at a 5-molar equivalent concentration in a buffering solution at pH 5.0, and the absorption spectra were measured. The malvin concentration was 0.5 mM.

(67) TABLE-US-00001 TABLE 1 Absorption maximum (λmax) and hue angle (°) of malvin solution upon flavone C-glycoside addition Absorption maximum Hue (λmax) angle Isovitexin (apigenin 6-C-glucoside) 5 equiv. 576 nm 298° Vitexin (apigenin 8-C-glucoside) 5 equiv. 563 nm 312° Vicenin-2 (apigenin 6,8-di-C-glucoside) 5 equiv. 562 nm 314° Isoorientin (luteolin 6-C-glucoside) 5 equiv. 575 nm 299° Orientin (luteolin 8-C-glucoside) 5 equiv. 570 nm 307° — — 531

nm 334°

(68) Addition of a flavone C-glycoside increased the absorbance of the malvin solution and shifted the absorption maximum (λmax) toward the long wavelength end compared to malvin alone, regardless of which flavone C-glycoside was added. This effect was confirmed as a greater shift in absorption maximum toward the long wavelength end in the order: isovitexin >isoorientin >orientin >vitexin >vicenin-2. The hue angle was also confirmed to be smaller in the order: isovitexin >isoorientin >orientin >vitexin >vicenin-2. It was thus demonstrated that the copigment effect of flavone mono-C-glycosides is higher than that of flavone di-C-glycosides. In addition, it was shown that the copigment effect of flavone mono-C-glycosides is particularly high with flavone 6-C-glycosides.

Example 2: Transfer of *Campanula*-Derived F3′,5′H Gene, Torenia-Derived MT Gene, Licorice-Derived F2H Gene, Rice-Derived Codon Usage-Modified CGT Gene and *Lotus japonicus*-Derived FDH Gene into Rose Variety “Ocean Song”

(69) Plasmid pSPB6486 has pBINPLUS as the basic backbone, and contains the following four expression cassettes.

(70) (1) El.sub.235S promoter, *Campanula*-derived F3′,5′H full-length cDNA (SEQ ID NO: 1) and D8 terminator

(71) (2) El.sub.235S promoter, torenia-derived MT full-length cDNA (SEQ ID NO: 3) and NOS terminator

(72) (3) 35S promoter, licorice-derived F2H full-length cDNA (SEQ ID NO: 5) and perilla-derived AT terminator

(73) (4) 35S promoter, rice-derived codon usage-modified CGT full-length cDNA (SEQ ID NO: 7) and *Arabidopsis thaliana*-derived HSP terminator

(74) (5) 35S promoter, *Lotus japonicus*-derived FDH full-length cDNA (SEQ ID NO: 9) and *Arabidopsis thaliana*-derived HSP terminator

(75) This plasmid constitutively expresses the *Campanula* F3′,5′H gene, torenia MT gene, licorice F2H gene, rice codon usage-modified CGT gene and *Lotus japonicus* FDH gene in plants.

(76) The constructed plasmid pSPB6486 was introduced into the blue rose variety “Ocean Song”, and a total of 27 transformants were obtained. Upon pigment analysis, malvidin storage was confirmed in 26 transformants, with a maximum malvidin content of 74.5% (average: 57.0%). The flavone C-glycosides isovitexin (apigenin 6-C-glucoside), vitexin (apigenin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside), orientin (luteolin 8-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside) were also identified and quantified. Flavone C-glycosides were detected in all of the transformants in which malvidins were detected, with the flavone di-C-glycoside vicenin-2 as the major detected component. The amounts of production were greater than in the order: vicenin-2>isovitexin >vitexin >isoorientin >orientin, with a maximum total amount of 1.563 mg per 1 g of fresh petal weight. The total amount of flavone C-glycosides was more than about 10 times that of malvidins.

(77) The measured values for representative transformants are shown in Table 2 below.

(78) TABLE-US-00002 TABLE 2 Plant Mal Anthocyanidin (mg/g) Flavonol (mg/g) Flavone (mg/g) Flavone C-glycoside (mg/g) No. (%) Del Cya Pet Pel Mal M Q K Tri Lut Api Vic2 VX IVX Ori Iori Host 0.0 0.000 0.024 0.000 0.000 0.000 0.000 2.883 0.586 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 1 70.8 0.024 0.000 0.009 0.000 0.080 1.225 0.077 0.023 0.000 0.013 0.010 0.187 0.205 0.505 0.009 0.073 2 66.2 0.025 0.000 0.007 0.000 0.062 2.453 0.181 0.020 0.009 0.047 0.029 0.743 0.109 0.319 0.027 0.077 3 54.6 0.022 0.006 0.008 0.000 0.050 1.058 1.381 0.515 0.000 0.000 0.000 0.896 0.093 0.219 0.000 0.015 4 52.7 0.020 0.003 0.007 0.000 0.035 0.858 0.482 0.178 0.000 0.003 0.006 0.429 0.174 0.354 0.007 0.035 5 64.0 0.028 0.000 0.009 0.000 0.065 2.045 0.168 0.063 0.000 0.000 0.000 0.586 0.130 0.299 0.002 0.030 6 67.7 0.028 0.000 0.007 0.000 0.074 1.593 0.455 0.115 0.011 0.010 0.009 0.795 0.126 0.355 0.018 0.050 7 72.1 0.027 0.000 0.008 0.000 0.089 2.753 0.526 0.183 0.006 0.004 0.004 0.905 0.093 0.262 0.000 0.028

8 54.7 0.019 0.007 0.005 0.000 0.049 1.010 2.128 0.418 0.018 0.002 0.005 0.945 0.226 0.325 0.000 0.021 9 39.1 0.018 0.009 0.004 0.000 0.025 0.679 1.438 0.322 0.005 0.002 0.002 0.672 0.246 0.283 0.007 0.020 10 39.8 0.013 0.009 0.005 0.000 0.021 0.447 1.129 0.586 0.000 0.000 0.002 0.432 0.080 0.179 0.000 0.010 11 45.9 0.011 0.011 0.006 0.000 0.034 0.914 3.471 1.481 0.000 0.000 0.000 0.839 0.050 0.118 0.000 0.003 12 45.4 0.022 0.005 0.007 0.000 0.032 0.803 0.992 0.418 0.000 0.004 0.005 0.511 0.142 0.354 0.000 0.024 13 62.8 0.032 0.000 0.008 0.000 0.067 1.830 0.210 0.059 0.018 0.023 0.015 0.910 0.000 0.249 0.000 0.049 14 65.6 0.024 0.000 0.007 0.000 0.060 1.942 0.258 0.078 0.011 0.019 0.015 1.047 0.056 0.305 0.016 0.054 15 52.9 0.042 0.002 0.009 0.000 0.062 0.993 0.377 0.100 0.000 0.004 0.005 0.396 0.197 0.450 0.000 0.050 16 63.9 0.031 0.000 0.007 0.000 0.067 2.742 0.221 0.056 0.009 0.030 0.018 0.591 0.063 0.285 0.022 0.058 17 63.5 0.016 0.000 0.005 0.000 0.037 1.767 0.102 0.013 0.009 0.051 0.029 0.621 0.147 0.242 0.022 0.061 18 74.5 0.029 0.000 0.009 0.000 0.109 2.078 0.187 0.029 0.017 0.030 0.015 0.604 0.051 0.276 0.021 0.061 19 55.4 0.019 0.009 0.006 0.000 0.059 0.937 1.893 0.502 0.008 0.001 0.000 1.002 0.078 0.218 0.000 0.007 20 38.5 0.006 0.010 0.002 0.000 0.019 0.480 2.621 0.468 0.007 0.001 0.003 0.890 0.147 0.247 0.000 0.012 21 47.7 0.025 0.000 0.009 0.000 0.031 2.684 0.376 0.102 0.008 0.019 0.018 0.700 0.147 0.303 0.017 0.048 22 39.5 0.011 0.013 0.003 0.000 0.026 0.497 2.333 0.524 0.000 0.004 0.008 0.720 0.224 0.346 0.009 0.030 23 65.3 0.019 0.001 0.007 0.000 0.055 1.615 1.007 0.223 0.005 0.001 0.000 0.828 0.101 0.207 0.000 0.013 24 70.0 0.028 0.000 0.008 0.000 0.087 2.776 0.531 0.166 0.000 0.001 0.000 0.913 0.055 0.178 0.000 0.012 25 66.2 0.014 0.003 0.007 0.000 0.058 1.428 1.898 0.525 0.000 0.008 0.014 0.907 0.255 0.368 0.000 0.034 26 42.7 0.036 0.017 0.011 0.000 0.063 0.991 2.495 0.457 0.000 0.000 0.000 0.529 0.030 0.094 0.000 0.000 Host: Ocean Song Del: delphinidin, Cya: cyanidin, Pet: petunidin, Pel: pelargonidin, Mal: malvidin M: myricetin, Q: quercetin, K: kaempferol Tri: tricetin, Lut: luteolin, Api: apigenin, Vic2: vicenin-2, VX: vitexin, IVX: isovitexin, Ori: orientin, Iori: isoorientin Mal(%): Proportion of malvidin in total anthocyanidins

Example 3: Transfer of *Campanula*-Derived F3′,5′H Gene, Torenia-Derived MT Gene, Licorice-Derived F2H Gene, Buckwheat-Derived Codon Usage-Modified CGT Gene and *Lotus japonicus*-Derived FDH Gene into Rose Variety “Ocean Song”

(79) Plasmid pSPB7473 has pBINPLUS as the basic backbone, and contains the following four expression cassettes.

(80) (1) El.sub.235S promoter, *Campanula*-derived F3′,5′H full-length cDNA (SEQ ID NO: 1) and D8 terminator

(81) (2) El.sub.235S promoter, torenia-derived MT full-length cDNA (SEQ ID NO: 3) (*Arabidopsis thaliana* HSPRO gene-derived 5′-UTR (SEQ ID NO: 13) added to the 5′-position end) and *Arabidopsis thaliana*-derived HSP terminator

(82) (3) El.sub.235S promoter, licorice-derived F2H full-length cDNA (SEQ ID NO: 5) and *perilla*-derived AT terminator

(83) (4) El.sub.235S promoter, buckwheat-derived codon usage-modified CGT full-length cDNA (SEQ ID NO: 11) (*Arabidopsis thaliana* HSPRO gene-derived 5′-UTR (SEQ ID NO: 13) added to the 5′-position end) and *Arabidopsis thaliana*-derived HSP terminator

(84) (5) El.sub.235S promoter, *Lotus japonicus*-derived FDH full-length cDNA (SEQ ID NO: 9) and *Arabidopsis thaliana*-derived HSP terminator

(85) This plasmid constitutively expresses the *Campanula* F3′,5′H gene, torenia MT gene, licorice F2H gene, buckwheat codon usage-modified CGT gene and *Lotus japonicus* FDH gene in plants.

(86) The constructed plasmid pSPB7473 was introduced into the blue rose variety “Ocean Song”, and a total of 35 transformants were obtained. Upon pigment analysis, malvidin storage was confirmed in 21 transformants, with a maximum malvidin content of 20.8% (average: 9.1%). The flavone C-glycosides isovitexin (apigenin 6-C-glucoside), vitexin (apigenin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside), orientin (luteolin 8-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside) were also identified and quantified. Flavone C-glycosides were detected in all of

the transformants in which malvidins were detected, with the flavone 6-C-glycoside isovitexin as the major detected component. The amounts of production were greater in the order isovitexin >vitexin >vicenin-2>isoorientin >orientin, and the maximum total amount was 7.418 mg per 1 g of fresh petal weight, indicating a very high content. In addition, in comparison to the OS/6486 line described in Example 2, the total amount of flavone C-glycosides was a high content of 3 mg or greater per 1 g of fresh petal weight in most of the transformants, which was at least about 100 times compared to delphinidins.

(87) The measured values for representative transformants are shown in Table 3 below.

(88) TABLE-US-00003 TABLE 3 Total flavone C- glyco- sides Anthocyanidin Flavonol Flavone Flavone C-glycoside (mg/g Plant (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) Mal fresh No. Del Cya Pet Pel Mal M Q K Tri Lut Api Vic2 VX IVX Ori Iori (%) weight) Host 0.000 0.024 0.000 0.000 0.000 0.000 2.883 0.586 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 — — 1 0.053 0.001 0.005 0.000 0.003 1.261 0.220 0.044 0.029 0.000 0.000 0.922 1.500 1.927 0.083 0.169 5.4 4.601 2 0.065 0.000 0.007 0.000 0.003 2.520 0.130 0.051 0.042 0.000 0.000 1.773 2.140 2.893 0.141 0.288 4.2 7.235 3 0.086 0.004 0.009 0.000 0.005 1.459 0.419 0.093 0.000 0.000 0.000 1.160 1.777 2.203 0.082 0.165 4.8 5.387 4 0.054 0.000 0.008 0.000 0.009 2.885 0.168 0.032 0.000 0.000 0.000 0.041 0.000 0.000 0.000 0.000 13.0 0.041 5 0.063 0.000 0.009 0.000 0.007 2.903 0.186 0.055 0.041 0.000 0.000 1.787 2.255 2.962 0.138 0.276 8.7 7.418 6 0.009 0.004 0.002 0.000 0.002 1.096 2.107 0.345 0.026 0.000 0.026 1.093 1.774 2.711 0.095 0.177 9.6 5.849 7 0.012 0.008 0.002 0.000 0.001 0.683 1.642 0.173 0.025 0.000 0.015 0.800 1.192 1.648 0.063 0.138 6.2 3.840 8 0.011 0.001 0.003 0.000 0.002 1.737 0.984 0.164 0.029 0.000 0.010 0.990 1.582 2.389 0.100 0.195 12.5 5.256 9 0.026 0.016 0.002 0.000 0.001 0.723 1.928 0.181 0.000 0.000 0.012 0.796 1.080 1.320 0.000 0.114 3.0 3.310 10 0.022 0.008 0.002 0.000 0.002 1.017 1.325 0.309 0.000 0.000 0.000 1.394 1.442 2.123 0.070 0.136 5.5 5.165 11 0.040 0.000 0.004 0.000 0.002 1.706 0.177 0.060 0.019 0.000 0.000 0.851 1.086 1.541 0.068 0.129 4.1 3.674 12 0.035 0.000 0.003 0.000 0.002 1.898 0.219 0.069 0.023 0.000 0.000 1.000 1.328 1.892 0.082 0.165 4.6 4.466 13 0.031 0.000 0.004 0.000 0.003 0.888 0.041 0.012 0.027 0.004 0.000 1.120 1.482 1.939 0.091 0.194 7.8 4.826 14 0.040 0.000 0.005 0.000 0.005 0.980 0.048 0.017 0.024 0.000 0.000 1.153 1.387 1.757 0.087 0.183 9.1 4.568 15 0.027 0.000 0.007 0.000 0.009 1.273 0.108 0.030 0.000 0.000 0.000 0.533 1.386 1.402 0.059 0.122 20.8 3.501 16 0.020 0.000 0.005 0.000 0.006 1.567 0.129 0.045 0.025 0.000 0.008 0.610 1.523 1.819 0.084 0.165 18.9 4.200 17 0.018 0.051 0.003 0.000 0.004 0.188 1.761 0.118 0.000 0.000 0.000 0.705 0.409 0.632 0.016 0.043 5.1 1.806 18 0.014 0.035 0.002 0.000 0.004 0.242 2.042 0.265 0.010 0.000 0.000 1.008 0.682 1.022 0.034 0.066 7.7 2.813 19 0.021 0.028 0.005 0.000 0.014 0.339 1.434 0.167 0.012 0.000 0.000 0.935 0.919 1.241 0.037 0.081 19.7 3.214 20 0.014 0.033 0.002 0.000 0.006 0.236 1.697 0.204 0.000 0.000 0.000 1.155 0.839 1.265 0.036 0.086 10.0 3.381 21 0.045 0.001 0.006 0.000 0.006 1.641 0.302 0.087 0.018 0.000 0.000 0.987 1.066 1.296 0.051 0.099 10.9 3.499 Average 9.1 4.193 S.D. 5.3 1.663 Host: Ocean Song Del: delphinidin, Cya: cyanidin, Pet: petunidin, Pel: pelargonidin, Mal: malvidin M: myricetin, Q: quercetin, K: kaempferol Tri: tricetin, Lut: luteolin, Api: apigenin, Vic2: vicenin-2, VX: vitexin, IVX: isovitexin, Ori: orientin, Iori: isoorientin Mal(%): Proportion of malvidin in total anthocyanidins

Example 4: Transfer of *Campanula*-Derived F3′,5′H Gene, Torenia-Derived MT Gene, Licorice-Derived F2H Gene, Buckwheat-Derived CGT Gene and *Lotus japonicus*-Derived FDH Gene into Rose Variety “Ocean Song”

(89) Plasmid pSPB7472 has pBINPLUS as the basic backbone, and contains the following four expression cassettes.

(90) (1) El.sub.235S promoter, *Campanula*-derived F3′,5′H full-length cDNA (SEQ ID NO: 1) and D8 terminator

(91) (2) El.sub.235S promoter, torenia-derived MT full-length cDNA (SEQ ID NO: 3) (*Arabidopsis thaliana* HSPRO gene-derived 5′-UTR (SEQ ID NO: 13) added to the 5′-position end) and

Arabidopsis thaliana-derived HSP terminator
(92) (3) El.sub.235S promoter, licorice-derived F2H full-length cDNA (SEQ ID NO: 5) and *perilla*-derived AT terminator
(93) (4) El.sub.235S promoter, buckwheat-derived CGT full-length cDNA (SEQ ID NO: 14) (*Arabidopsis thaliana* HSPRO gene-derived 5′-UTR (SEQ ID NO: 13)) added to the 5′-position end) and *Arabidopsis thaliana*-derived HSP terminator
(94) (5) El.sub.235S promoter, *Lotus japonicus*-derived FDH full-length cDNA (SEQ ID NO: 9) and *Arabidopsis thaliana*-derived HSP terminator
(95) This plasmid constitutively expresses the *Campanula* F3′,5′H gene, torenia MT gene, licorice F2H gene, buckwheat CGT gene and *Lotus japonicus* FDH gene in plants.
(96) The constructed plasmid pSPB7472 was introduced into the blue rose variety “Ocean Song”, and a total of 33 transformants were obtained. The flavone C-glycosides isovitexin (apigenin 6-C-glucoside), vitexin (apigenin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside), orientin (luteolin 8-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside), and the anthocyanidins delphinidin, cyanidin, petunidin, pelargonidin and malvidin, were then identified and quantified. As a result, accumulation of flavone C-glycosides and malvidin was confirmed in 11 transformants. The average content of flavone C-glycosides was 3.75 mg per 1 g of fresh petal weight, with the flavone 6-C-glycoside isovitexin detected as the main component. The malvidin content was a maximum of 15.6% (average: 8.8%).
(97) Thus, the average content of flavone C-glycosides per 1 g of fresh petal weight was higher in the OS/7473 line described in Example 3, at 4.19 mg. In other words, it is possible to produce flavone C-glycosides more efficiently with the codon-modified CGT gene than with the original buckwheat-derived CGT gene.
(98) The measured values for representative transformants are shown in Table 4 below.
(99) TABLE-US-00004 TABLE 4 Total flavone Anthocyanidin Flavonol Flavone Flavone C-glycoside C-gly cosides Plant (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) Mal (mg/g fresh No. Del Cya Pet Pel Mal M Q K Tri Lut Api Vic2 VX IVX Ori Iori (%) weight) Host 0.000 0.024 0.000 0.000 0.000 0.000 2.883 0.586 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 — — 1 0.120 0.000 0.027 0.000 0.027 2.784 0.211 0.029 0.000 0.000 0.000 0.061 0.000 0.000 0.000 0.000 15.6 0.061 2 0.041 0.000 0.007 0.000 0.003 2.485 0.369 0.094 0.036 0.000 0.000 1.010 1.873 2.654 0.107 0.221 6.8 5.864 3 0.100 0.002 0.008 0.000 0.002 1.632 0.316 0.055 0.000 0.000 0.000 1.244 2.093 2.482 0.103 0.204 1.9 6.127 4 0.025 0.000 0.002 0.000 0.001 1.916 0.221 0.060 0.026 0.000 0.000 0.872 1.545 2.228 0.104 0.200 4.1 4.950 5 0.031 0.000 0.005 0.000 0.003 2.125 0.298 0.076 0.021 0.000 0.000 0.847 1.400 1.937 0.075 0.156 6.7 4.415 6 0.039 0.000 0.008 0.000 0.005 1.926 0.251 0.072 0.000 0.000 0.000 0.717 1.292 1.731 0.070 0.143 8.9 3.953 7 0.043 0.000 0.004 0.000 0.003 1.117 0.126 0.031 0.024 0.000 0.000 1.304 1.365 1.781 0.082 0.177 5.3 4.710 8 0.053 0.002 0.010 0.000 0.010 0.968 0.143 0.023 0.000 0.000 0.000 0.982 1.064 1.495 0.062 0.129 13.2 3.732 9 0.016 0.000 0.005 0.000 0.004 1.759 0.175 0.083 0.000 0.000 0.000 0.661 0.941 2.106 0.100 0.174 14.9 3.982 10 0.014 0.000 0.004 0.000 0.003 1.618 0.148 0.088 0.000 0.000 0.000 0.651 0.820 1.741 0.000 0.153 13.4 3.366 11 0.011 0.000 0.002 0.000 0.001 0.292 0.000 0.000 0.332 0.433 0.082 0.095 0.000 0.000 0.000 0.000 6.3 0.095 Average 8.8 3.750 S.D. 4.7 2.003 Host: Ocean Song Del: delphinidin, Cya: cyanidin, Pet: petunidin, Pel: pelargonidin, Mal: malvidin M: myricetin, Q: quercetin, K: kaempferol Tri: tricetin, Lut: luteolin, Api: apigenin, Vic2: vicenin-2, VX: vitexin, IVX: isovitexin, Ori: orientin, Iori: isoorientin Mal(%): Proportion of malvidin in total anthocyanidins

Example 5: Transfer of *Campanula*-Derived F3′,5′H Gene, Torenia-Derived MT Gene, Licorice-Derived F2H Gene, Buckwheat-Derived CGT Gene and *Lotus japonicus*-Derived FDH Gene into Rose Variety “Ocean Song”

(100) Plasmid pSPB7808 has pBINPLUS as the basic backbone, and contains the following four expression cassettes.

(101) (1) El.sub.235S promoter, *Campanula*-derived F3′,5′H full-length cDNA (SEQ ID NO: 1) and D8 terminator
(102) (2) El.sub.235S promoter, torenia-derived MT full-length cDNA (SEQ ID NO: 3) and *Arabidopsis thaliana*-derived HSP terminator
(103) (3) El.sub.235S promoter, licorice-derived F2H full-length cDNA (SEQ ID NO: 5) and *perilla*-derived AT terminator
(104) (4) El.sub.235S promoter, buckwheat-derived CGT full-length cDNA (SEQ ID NO: 14) (*Arabidopsis thaliana* alcohol dehydrogenase (ADH) gene-derived 5′-UTR (SEQ ID NO: 15) added to the 5′-position end) and *Arabidopsis thaliana*-derived HSP terminator
(105) (5) El.sub.235S promoter, *Lotus japonicus*-derived FDH full-length cDNA (SEQ ID NO: 9) and *Arabidopsis thaliana*-derived HSP terminator
(106) This plasmid constitutively expresses the *Campanula* F3′,5′H gene, torenia MT gene, licorice F2H gene, buckwheat CGT gene and *Lotus japonicus* FDH gene in plants.
(107) The constructed plasmid pSPB7808 was introduced into the blue rose variety “Ocean Song”, and a total of 65 transformants were obtained. The flavone C-glycosides isovitexin (apigenin 6-C-glucoside), vitexin (apigenin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside), orientin (luteolin 8-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside), and the anthocyanidins delphinidin, cyanidin, petunidin, pelargonidin and malvidin, were then identified and quantified. As a result, accumulation of flavone C-glycosides and malvidin was confirmed in 32 transformants. The mean content of flavone C-glycosides was 3.00 mg per 1 g of fresh petal weight, with the flavone 6-C-glycoside isovitexin detected as the main component. The malvidin content was a maximum of 69.2% (average: 43.9%).
(108) The measured values for representative transformants are shown in Table 5 below.
(109) TABLE-US-00005 TABLE 5 Total flavone C- glycosides Anthocyanidin Flavonol Flavone Flavone C-glycoside (mg/g Plant (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) Mal fresh No. Del Cya Pet Pel Mal M Q K Tri Lut Api Vic2 VX IVX Ori Iori (%) weight) Host 0.000 0.024 0.000 0.000 0.000 0.000 2.883 0.586 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 — — 1 0.052 0.000 0.004 0.000 0.002 0.755 0.000 0.000 0.074 0.174 0.086 0.329 1.123 2.417 0.145 0.285 3.0 4.299 2 0.026 0.002 0.020 0.000 0.068 1.448 0.321 0.206 0.000 0.000 0.000 0.560 0.587 1.380 0.000 0.056 57.1 2.583 3 0.021 0.000 0.010 0.000 0.026 1.569 0.271 0.252 0.000 0.000 0.000 0.898 0.878 1.945 0.051 0.108 46.7 3.880 4 0.022 0.000 0.009 0.000 0.023 1.549 0.340 0.265 0.000 0.000 0.000 0.867 0.874 1.792 0.044 0.090 42.5 3.667 5 0.006 0.000 0.004 0.000 0.005 0.775 0.000 0.000 0.027 0.146 0.120 0.238 0.431 1.005 0.063 0.115 34.4 1.851 6 0.006 0.000 0.003 0.000 0.002 0.747 0.000 0.000 0.025 0.110 0.083 0.237 0.389 0.907 0.065 0.116 18.8 1.715 7 0.007 0.000 0.003 0.000 0.007 1.014 0.064 0.028 0.029 0.148 0.100 0.410 0.596 1.338 0.099 0.176 41.0 2.619 8 0.007 0.000 0.003 0.000 0.006 1.134 0.080 0.025 0.034 0.183 0.127 0.354 0.638 1.423 0.101 0.179 36.9 2.696 9 0.009 0.000 0.004 0.000 0.011 1.713 0.303 0.127 0.000 0.000 0.000 0.617 0.682 1.498 0.052 0.101 46.6 2.949 10 0.006 0.000 0.005 0.000 0.016 1.359 0.292 0.128 0.000 0.000 0.000 0.545 0.581 1.387 0.000 0.068 60.2 2.582 11 0.006 0.000 0.003 0.000 0.003 0.809 0.054 0.020 0.025 0.124 0.101 0.184 0.501 1.138 0.074 0.130 26.2 2.027 12 0.004 0.000 0.003 0.000 0.005 0.692 0.065 0.031 0.020 0.122 0.107 0.186 0.484 1.109 0.065 0.115 42.3 1.959 13 0.006 0.010 0.003 0.000 0.015 0.403 1.049 0.445 0.000 0.000 0.000 0.292 0.144 0.260 0.000 0.000 36.1 0.695 14 0.005 0.002 0.002 0.000 0.026 0.594 0.363 0.193 0.000 0.000 0.000 0.403 0.216 0.545 0.000 0.000 69.2 1.165 15 0.008 0.002 0.003 0.000 0.027 1.171 1.128 0.196 0.000 0.000 0.000 1.749 1.080 1.891 0.000 0.101 61.8 4.821 16 0.006 0.000 0.004 0.000 0.005 0.880 0.064 0.022 0.030 0.162 0.142 0.286 0.633 1.469 0.088 0.141 30.5 2.617 17 0.015 0.000 0.009 0.000 0.016 2.291 0.190 0.064 0.065 0.322 0.220 0.440 1.307 3.073 0.205 0.381 40.4 5.406 18 0.008 0.000 0.004 0.000 0.003 1.684 0.157 0.031 0.045 0.155 0.168 0.543 1.149 2.038 0.131 0.238 22.3 4.099 19 0.005 0.000 0.003 0.000 0.007 0.712 0.074 0.026 0.000 0.000 0.000 0.558 0.346 0.816 0.000 0.067 48.0 1.788 20 0.005 0.000 0.003

0.000 0.007 0.774 0.080 0.027 0.000 0.000 0.000 0.479 0.356 0.848 0.000 0.068 47.5 1.751 21 0.005 0.000 0.003 0.000 0.009 0.614 0.054 0.000 0.000 0.000 0.000 0.427 0.294 0.699 0.000 0.056 51.1 1.477 22 0.005 0.000 0.005 0.000 0.013 1.117 0.179 0.075 0.000 0.000 0.000 0.481 0.414 1.023 0.000 0.063 57.5 1.981 23 0.007 0.000 0.006 0.000 0.019 1.374 0.257 0.102 0.000 0.000 0.000 0.551 0.478 1.178 0.000 0.068 59.9 2.275 24 0.006 0.000 0.004 0.000 0.014 1.181 0.228 0.082 0.000 0.000 0.000 0.433 0.403 0.984 0.000 0.057 58.9 1.877 25 0.010 0.000 0.005 0.000 0.011 0.641 0.096 0.019 0.000 0.000 0.000 1.121 0.642 1.207 0.000 0.122 42.6 3.092 26 0.008 0.000 0.005 0.000 0.013 1.270 0.149 0.066 0.000 0.000 0.000 1.751 1.287 1.468 0.000 0.143 50.8 4.649 27 0.020 0.000 0.009 0.000 0.040 0.471 0.095 0.000 0.000 0.000 0.000 1.635 1.750 2.498 0.000 0.176 57.9 6.059 28 0.021 0.000 0.010 0.000 0.027 0.947 0.259 0.060 0.000 0.000 0.000 1.081 0.848 1.753 0.000 0.128 46.7 3.810 29 0.018 0.002 0.009 0.000 0.009 0.589 0.079 0.017 0.000 0.065 0.060 0.567 0.640 1.344 0.000 0.155 23.2 2.706 30 0.010 0.000 0.005 0.000 0.011 1.521 0.330 0.148 0.020 0.014 0.032 1.055 0.877 1.639 0.000 0.124 41.9 3.694 31 0.008 0.000 0.005 0.000 0.014 2.170 0.595 0.163 0.000 0.000 0.000 1.406 1.255 2.150 0.000 0.157 51.5 4.969 32 0.006 0.000 0.003 0.000 0.009 1.998 0.519 0.142 0.000 0.000 0.000 1.156 1.098 1.854 0.068 0.143 49.6 4.318 Average 43.9 3.002 S.D. 14.4 1.329 Host: Ocean Song Del: delphinidin, Cya: cyanidin, Pet: petunidin, Pel: pelargonidin, Mal: malvidin M: myricetin, Q: quercetin, K: kaempferol Tri: tricetin, Lut: luteolin, Api: apigenin, Vic2: vicenin-2, VX: vitexin, IVX: isovitexin, Ori: orientin, Iori: isoorientin Mal(%): Proportion of malvidin in total anthocyanidins

Example 6: Transfer of *Campanula*-Derived F3′,5′H Gene, Torenia-Derived MT Gene, Licorice-Derived F2H Gene, Buckwheat-Derived Codon Usage-Modified CGT Gene and *Lotus japonicus*-Derived FDH Gene into Rose Variety “Ocean Song”

(110) Plasmid pSPB7809 has pBINPLUS as the basic backbone, and contains the following four expression cassettes.

(111) (1) El.sub.235S promoter, *Campanula*-derived F3′,5′H full-length cDNA (SEQ ID NO: 1) and D8 terminator

(112) (2) El.sub.235S promoter, torenia-derived MT full-length cDNA (SEQ ID NO: 3) and *Arabidopsis thaliana*-derived HSP terminator

(113) (3) El.sub.235S promoter, licorice-derived F2H full-length cDNA (SEQ ID NO: 5) and *perilla*-derived AT terminator

(114) (4) El.sub.235S promoter, buckwheat-derived codon usage-modified CGT full-length cDNA (SEQ ID NO: 11) (*Arabidopsis thaliana* alcohol dehydrogenase (ADH) gene-derived 5′-UTR (SEQ ID NO: 15) added to the 5′-position end) and *Arabidopsis thaliana*-derived HSP terminator

(115) (5) El.sub.235S promoter, *Lotus japonicus*-derived FDH full-length cDNA (SEQ ID NO: 9) and *Arabidopsis thaliana*-derived HSP terminator

(116) This plasmid constitutively expresses the *Campanula* F3′,5′H gene, torenia MT gene, licorice F2H gene, buckwheat codon usage-modified CGT gene and *Lotus japonicus* FDH gene in plants.

(117) The constructed plasmid pSPB7809 was introduced into the blue rose variety “Ocean Song”, and a total of 143 transformants were obtained. The flavone C-glycosides isovitexin (apigenin 6-C-glucoside), vitexin (apigenin 8-C-glucoside), isoorientin (luteolin 6-C-glucoside), orientin (luteolin 8-C-glucoside) and vicenin-2 (apigenin 6,8-di-C-glucoside), and the anthocyanidins delphinidin, cyanidin, petunidin, pelargonidin and malvidin, were then identified and quantified. As a result, accumulation of flavone C-glycosides was confirmed in 58 transformants. The mean content of flavone C-glycosides was 3.24 mg per 1 g of fresh petal weight, with the flavone 6-C-glycoside isovitexin detected as the main component. The malvidin content was a maximum of 80.3% (average: 46.6%).

(118) Thus, the average content of flavone C-glycosides per 1 g of fresh petal weight was higher in the OS/7809 line described in the Examples. Similar to the results obtained in Examples 3 and 4, it was shown to be possible to produce flavone C-glycosides more efficiently with the codon-modified CGT gene than with the original buckwheat-derived CGT gene.

(119) The measured values for representative transformants are shown in Table 6 below.

(120) TABLE-US-00006 TABLE 6 Total flavone C- glycosides Anthocyanidin Flavonol Flavone Flavone C-glycoside (mg/g Plant (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) (mg/g fresh weight) Mal fresh No. Del Cya Pet Pel Mal M Q K Tri Lut Api Vic2 VX IVX Ori Iori (%) weight) Host 0.000 0.024 0.000 0.000 0.000 0.000 2.883 0.586 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 — — 1 0.019 0.000 0.003 0.000 0.002 0.576 0.068 0.020 0.083 0.137 0.071 0.445 1.009 2.121 0.136 0.280 7.0 3.991 2 0.011 0.000 0.005 0.000 0.033 0.865 0.091 0.042 0.000 0.000 0.000 1.133 0.673 1.375 0.047 0.106 67.1 3.335 3 0.011 0.000 0.005 0.000 0.028 1.183 0.119 0.068 0.000 0.000 0.000 0.954 0.602 1.284 0.053 0.096 63.9 2.988 4 0.009 0.000 0.005 0.000 0.017 1.332 0.177 0.093 0.000 0.000 0.000 0.942 0.498 1.150 0.041 0.089 54.4 2.720 5 0.015 0.000 0.007 0.000 0.018 1.521 0.209 0.107 0.000 0.000 0.000 1.223 0.748 1.707 0.062 0.139 45.8 3.879 6 0.013 0.000 0.007 0.000 0.017 1.007 0.131 0.064 0.000 0.000 0.000 0.844 0.523 1.181 0.047 0.101 46.1 2.694 7 0.017 0.000 0.006 0.000 0.018 1.852 0.363 0.200 0.000 0.000 0.000 0.759 0.711 1.482 0.042 0.091 44.5 3.083 8 0.009 0.000 0.006 0.000 0.018 1.395 0.253 0.130 0.000 0.000 0.000 0.819 0.819 1.790 0.052 0.113 53.2 3.593 9 0.007 0.000 0.002 0.000 0.006 0.694 0.000 0.000 0.057 0.254 0.122 0.381 0.470 1.075 0.105 0.187 38.8 2.219 10 0.011 0.000 0.003 0.000 0.003 0.711 0.000 0.000 0.062 0.222 0.099 0.365 0.533 1.243 0.117 0.214 18.9 2.472 11 0.010 0.000 0.005 0.000 0.007 0.772 0.000 0.000 0.023 0.104 0.090 0.481 0.451 1.070 0.062 0.122 32.1 2.186 12 0.009 0.000 0.005 0.000 0.012 1.093 0.121 0.066 0.000 0.000 0.000 0.618 0.508 1.105 0.051 0.078 47.4 2.360 13 0.006 0.000 0.004 0.000 0.008 0.858 0.070 0.027 0.000 0.000 0.000 1.033 0.874 1.896 0.071 0.163 44.1 4.038 14 0.035 0.003 0.005 0.000 0.003 1.213 0.803 0.673 0.000 0.000 0.000 0.599 0.064 0.263 0.000 0.000 5.7 0.926 15 0.011 0.000 0.003 0.000 0.002 0.921 0.000 0.000 0.066 0.243 0.117 0.387 0.685 1.543 0.129 0.218 15.2 2.962 16 0.005 0.000 0.003 0.000 0.011 0.540 0.087 0.075 0.000 0.000 0.000 0.841 0.569 1.286 0.000 0.085 58.1 2.781 17 0.004 0.000 0.002 0.000 0.007 0.878 0.000 0.025 0.044 0.202 0.135 0.451 0.619 1.438 0.114 0.198 53.0 2.820 18 0.004 0.000 0.003 0.000 0.019 1.616 0.106 0.000 0.000 0.000 0.000 0.722 0.515 1.201 0.043 0.115 73.7 2.595 19 0.007 0.000 0.005 0.000 0.043 2.309 0.235 0.097 0.000 0.000 0.000 0.655 0.625 1.409 0.000 0.097 77.2 2.786 20 0.016 0.000 0.005 0.000 0.009 0.771 0.079 0.035 0.056 0.155 0.119 0.532 0.812 1.796 0.100 0.193 30.2 3.433 21 0.016 0.000 0.008 0.000 0.011 0.788 0.061 0.027 0.062 0.123 0.081 0.749 1.191 2.623 0.126 0.262 32.0 4.951 22 0.010 0.000 0.005 0.000 0.007 0.884 0.055 0.000 0.030 0.116 0.085 0.514 0.679 1.530 0.085 0.156 32.8 2.964 23 0.006 0.000 0.003 0.000 0.031 0.947 0.171 0.082 0.000 0.000 0.000 1.412 0.844 2.030 0.058 0.113 76.8 4.457 24 0.011 0.000 0.004 0.000 0.028 1.048 0.173 0.091 0.000 0.000 0.000 0.959 0.608 1.396 0.048 0.102 65.0 3.113 25 0.015 0.000 0.003 0.000 0.005 1.028 0.000 0.000 0.050 0.134 0.071 0.336 0.997 1.946 0.139 0.245 21.8 3.664 26 0.006 0.000 0.004 0.000 0.010 0.443 0.000 0.000 0.165 0.397 0.114 0.330 0.000 0.418 0.045 0.110 49.1 0.903 27 0.005 0.000 0.003 0.000 0.008 0.513 0.000 0.000 0.124 0.604 0.259 0.267 0.157 0.397 0.040 0.074 47.5 0.935 28 0.007 0.000 0.004 0.000 0.012 0.363 0.000 0.000 0.092 0.327 0.104 0.207 0.103 0.291 0.000 0.066 51.5 0.667 29 0.015 0.000 0.011 0.000 0.026 1.208 0.161 0.059 0.000 0.000 0.000 1.856 0.926 2.140 0.079 0.229 49.6 5.229 30 0.013 0.000 0.009 0.000 0.020 1.138 0.097 0.039 0.000 0.000 0.000 1.499 0.819 1.941 0.070 0.198 47.5 4.526 31 0.008 0.000 0.004 0.000 0.007 1.354 0.112 0.036 0.019 0.093 0.090 0.395 0.752 1.661 0.089 0.154 35.0 3.051 32 0.005 0.000 0.003 0.000 0.009 1.819 0.287 0.181 0.000 0.000 0.000 1.436 1.025 2.410 0.064 0.194 52.6 5.128 33 0,007 0.000 0.005 0.000 0.012 1.233 0.148 0.072 0.000 0.000 0.000 0.975 0.604 1.441 0.055 0.109 51.4 3.185 34 0.005 0.000 0.003 0.000 0.011 2.099 0.333 0.154 0.000 0.000 0.000 1.263 0.733 1.737 0.056 0.116 57.4 3.905 35 0.014 0.000 0.009 0.000 0.024 1.248 0.283 0.131 0.000 0.000 0.000 1.183 0.760 1.884 0.043 0.146 51.2 4.016 36 0.014 0.000 0.003 0.000 0.002 0.375 0.034 0.000 0.033 0.092 0.049 0.350 0.479 1.099 0.079 0.134 10.6 2.142 37 0.010 0.000 0.002 0.000 0.001 0.705 0.077 0.021 0.031 0.068 0.056 0.426 0.620 1.405 0.075 0.135 9.3 2.660 38 0.003 0.000 0.002 0.000 0.017 1.087 0.153 0.066 0.000 0.000 0.000 1.481 0.744 1.612

0.050 0.109 80.3 3.995 39 0.001 0.000 0.000 0.000 0.004 0.988 0.469 0.204 0.000 0.000 0.000 0.805 0.661 1.547 0.043 0.073 72.8 3.128 40 0.003 0.003 0.002 0.000 0.011 0.992 1.325 0.414 0.000 0.000 0.000 1.271 0.853 1.871 0.047 0.097 51.4 4.140 41 0.006 0.000 0.002 0.000 0.017 1.430 0.621 0.293 0.000 0.000 0.000 0.812 0.741 1.633 0.053 0.087 68.3 3.325 42 0.004 0.000 0.002 0.000 0.014 1.223 0.356 0.195 0.000 0.000 0.000 0.981 0.861 1.781 0.000 0.076 70.9 3.699 43 0.008 0.000 0.004 0.000 0.023 1.211 0.512 0.350 0.000 0.000 0.000 1.026 0.935 2.040 0.000 0.081 66.6 4.081 44 0.009 0.000 0.005 0.000 0.008 1.333 0.147 0.037 0.053 0.203 0.088 0.187 0.337 0.796 0.079 0.139 36.4 1.537 45 0.020 0.001 0.011 0.000 0.016 1.182 0.000 0.022 0.093 0.348 0.140 0.255 0.349 0.777 0.084 0.163 32.9 1.629 46 0.016 0.000 0.009 0.000 0.016 1.232 0.125 0.038 0.074 0.289 0.114 0.160 0.390 0.844 0.088 0.159 39.2 1.641 47 0.004 0.000 0.003 0.000 0.005 0.824 0.057 0.000 0.033 0.090 0.088 0.555 1.502 3.439 0.152 0.276 42.9 5.924 48 0.007 0.000 0.005 0.000 0.016 1.366 0.349 0.123 0.000 0.000 0.000 0.672 0.682 1.510 0.000 0.086 56.4 2.951 49 0.009 0.000 0.005 0.000 0.017 1.407 0.382 0.138 0.000 0.000 0.000 0.594 0.654 1.456 0.039 0.084 55.1 2.828 50 0.006 0.000 0.003 0.000 0.033 2.879 0.358 0.065 0.000 0.000 0.000 1.522 0.782 1.397 0.000 0.135 77.9 3.836 51 0.018 0.000 0.008 0.000 0.020 0.765 0.147 0.027 0.000 0.000 0.000 1.224 0.584 1.174 0.000 0.108 43.7 3.090 52 0.021 0.000 0.010 0.000 0.027 1.359 0.357 0.183 0.000 0.000 0.000 2.105 0.689 1.831 0.000 0.109 46.5 4.734 53 0.009 0.000 0.006 0.000 0.016 1.485 0.248 0.073 0.000 0.000 0.000 1.808 0.630 1.256 0.000 0.113 52.7 3.807 54 0.005 0.000 0.003 0.000 0.018 1.235 0.610 0.220 0.000 0.000 0.000 1.640 1.201 1.893 0.000 0.084 68.3 4.818 55 0.007 0.000 0.004 0.000 0.006 1.082 0.086 0.019 0.034 0.076 0.096 0.569 1.517 2.754 0.133 0.262 34.9 5.234 56 0.008 0.000 0.003 0.000 0.002 0.998 0.000 0.000 0.072 0.253 0.166 0.457 0.667 1.062 0.092 0.183 18.7 2.461 57 0.017 0.000 0.005 0.000 0.006 1.031 0.000 0.000 0.078 0.120 0.064 0.543 1.090 1.621 0.151 0.286 21.9 3.691 58 0.010 0.000 0.006 0.000 0.014 1.598 0.292 0.111 0.000 0.000 0.000 1.511 0.931 1.176 0.000 0.098 46.7 3.717 Average 46.6 3.235 S.D. 18.9 1.143 Host: Ocean Song Del: delphinidin, Cya: cyanidin, Pet: petunidin, Pel: pelargonidin, Mal: malvidin M: myricetin, Q: quercetin, K: kaempferol Tri: tricetin, Lut: luteolin, Api: apigenin, Vic2: vicenin-2, VX: vitexin, IVX: isovitexin, Ori: orientin, Iori: isoorientin Mal(%): Proportion of malvidin in total anthocyanidins

Example 7: Evaluation of Flower Color of Flavone C-Glycoside-Containing Roses

(121) The transformants created in Examples 2 and 3 (with “Ocean Song” rose variety as host) were measured to determine the color shades of the respective petals using a CM-2022 spectrocolorimeter (Minolta) with a 10° visual field and a D65 light source, and analyzing with SpectraMagic™ color management software (Minolta).

(122) In comparing the average values of the hue angles, no differences in petal hue angle were found between the roses with buckwheat-derived (Example 3) and roses with rice-derived (Example 2) CGT genes. On the individual level, however, more transformants exhibited a hue angle of 315° or smaller among the roses with the transferred buckwheat-derived CGT gene, with one having been altered to the bluest color yet obtained, having a value of 294.5°. These results confirmed that using the buckwheat-derived CGT gene significantly increased the amount of flavone C-glycoside production, and especially the amount of mono-C-glycoside production, altering the petals to a blue color shade by their copresence with anthocyanins.

(123) The results are shown in Table 7.

(124) TABLE-US-00007 TABLE 7 Gene and flavonoid Hue angle composition (hue) Host Ocean Song Average: Stores cyanidin as main pigment, contains 362.57° absolutely no flavone C-glycoside (1) *Campanula* F3′,5′H Average: Has high storage of delphinidin as main 337.33°, pigment, contains absolutely no flavone bluest point: C-glycoside 333.15° Example (2) *Campanula* F3′,5′H + torenia MT + Average: 2 licorice F2H + rice CGT (codon usage modified) + *Lotus japonicus* FDH 320.16°, Has high storage of malvidin as main bluest point: pigment, contains flavone C-glycoside 318.89° Example (3) *Campanula* F3′,5′H + torenia MT + Average: 3 licorice F2H + buckwheat CGT (codon 320.19°, usage modified) + *Lotus japonicus* FDH bluest point: Has

high storage of delphinidin as main 294.53° pigment, contains flavone C-glycoside
SEQUENCE LISTING

Claims

1. A buckwheat-derived C-glucosyltransferase (CGT) gene or its homolog, wherein: the buckwheat-derived CGT gene or its homolog is a polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 11.
2. The buckwheat-derived CGT gene or its homolog according to claim 1, wherein the *Arabidopsis thaliana* alcohol dehydrogenase (ADH) gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or the *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) has been added.
3. A vector comprising a buckwheat-derived CGT gene or its homolog, wherein the buckwheat-derived CGT gene or its homolog is selected from the group consisting of: (a) a polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 11; (b) a polynucleotide that encodes a protein consisting of the amino acid sequence as set forth in SEQ ID NO: 12; and (c) a polynucleotide that encodes a protein having an amino acid sequence with at least 98% identity with respect to the amino acid sequence as set forth in SEQ ID NO: 12 and having the same activity as a protein encoded by the polynucleotide of (b), and wherein the vector further comprises a flavanone 2-hydroxylase (F2H) gene or its homolog, and a dehydratase (FDH) gene or its homolog.
4. The vector according to claim 3, wherein the *Arabidopsis thaliana* ADH gene-derived untranslated region (5′-UTR) (SEQ ID NO: 15) or the *Arabidopsis thaliana* HSPRO gene-derived untranslated region (5′-UTR) (SEQ ID NO: 13) has been added to the buckwheat-derived CGT gene or its homolog.
5. The vector according to claim 3, wherein the F2H gene or its homolog is selected from the group consisting of: (a) a polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 5; (b) a polynucleotide that encodes a protein consisting of the amino acid sequence as set forth in SEQ ID NO: 6; and (c) a polynucleotide that encodes a protein having an amino acid sequence with at least 98% identity with respect to the amino acid sequence as set forth in SEQ ID NO: 6 and having the same activity as a protein encoded by the polynucleotide of (b), and the FDH gene or its homolog is selected from the group consisting of: (a) a polynucleotide consisting of the nucleotide sequence as set forth in SEQ ID NO: 9; (b) a polynucleotide that encodes a protein consisting of the amino acid sequence as set forth in SEQ ID NO: 10; and (c) a polynucleotide that encodes a protein having an amino acid sequence with at least 98% identity with respect to the amino acid sequence as set forth in SEQ ID NO: 10 and having the same activity as a protein encoded by the polynucleotide of (3b).
6. A transgenic rose plant or its inbred or outbred progeny, a propagule thereof, a partial plant body thereof, a tissue or a cell of the transgenic rose plant thereof, a cut flower of the transgenic rose plant thereof comprising the buckwheat-derived CGT gene or its homolog according to claim 1.
7. The transgenic plant or its inbred or outbred progeny, a propagule thereof, a partial plant body thereof, a tissue or a cell of the transgenic rose plant thereof, a cut flower of the transgenic rose plant thereof according to claim 6, wherein the cut flower of the transgenic rose plant is a processed form selected from the group consisting of preserved, dried or resin sealed.
8. A method for creating transgenic rose plants with blue flower color, comprising a step of transferring the buckwheat-derived C-glucosyltransferase (CGT) gene according to claim 1 into a host rose plant to cause delphinidin-type anthocyanins and flavone mono-C-glycosides to coexist in the rose plant cells.
9. The method according to claim 8, wherein the flavone mono-C-glycoside is apigenin 6-C-glucoside, luteolin 6-C-glucoside, tricetin 6-C-glucoside, apigenin 8-C-glucoside, luteolin 8-C-

glucoside or tricetin 8-C-glucoside, or a derivative thereof.

10. The method according to claim 8, wherein the delphinidin-type anthocyanin is selected from the group consisting of malvidins, delphinidins, petunidins and their combinations.

11. A method for creating transgenic rose plants with blue flower color, comprising a step of transferring the vector according to claim 3 into a host plant to cause delphinidin-type anthocyanins and flavone mono-C-glycosides to coexist in the plant cells.