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(71) Applicant: **ISHIHARA SANGYO KAISHA, LTD.**
Osaka-shi,
Osaka 550-0002 (JP)

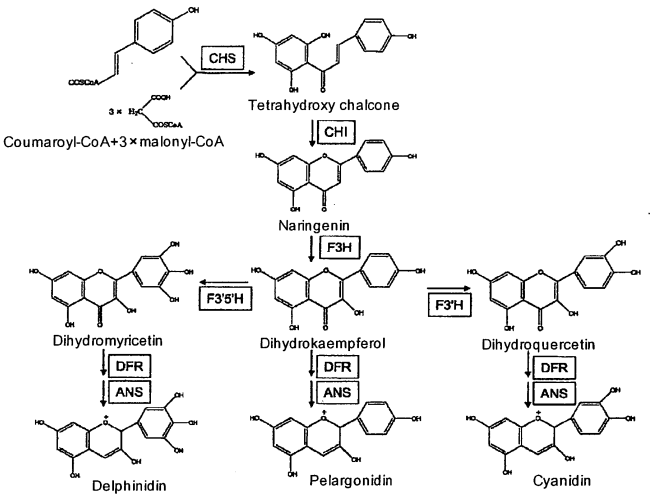
(72) Inventors:
- **YUKI, Shunji**
  Kusatsu-shi
  Shiga 525-0025 (JP)
- **ARAKI, Satoshi**
  Kusatsu-shi
  Shiga 525-0025 (JP)
- **SUZUKI, Takanori**
  Kusatsu-shi
  Shiga 525-0025 (JP)

(74) Representative: **Hartz, Nikolai et al**
Wächtershäuser & Hartz
Patentanwälte
Weinstrasse 8
80333 München (DE)

(54) DAYFLOWER FLAVONOID 3',5'-HYDROXYLASE GENE

(57) To provide a highly expressed F3'5'H gene, whereby a blue moth orchid can be produced and a method using the gene for producing Orchids having blue flowers. A gene encoding a flavonoid 3',5'-hydroxylase of *Commelina communis,* which has an amino acid sequence depicted in SEQ ID No: 2 or an amino acid sequence having at least 90% of homology to the amino acid sequence depicted in SEQ ID No: 2. A method for producing an orchid having a blue flower, which comprises transfecting a white moth orchid with the gene, a gene encoding a dihydroflavonol 4-reductase of *Torenia* or *Gerbera*; a gene encoding a flavanone 3-hydroxylase; and a gene encoding an anthocyanidin synthase and expressing the genes.

F i g. 1



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Description

TECHNICAL FIELD

[0001] The present invention relates to a method for producing a plant having a blue flower color by employing the gene recombination technology. Particularly, the present invention relates to a method for producing Orchids having a blue flower color by using a gene encoding a flavonoid-3',5'-hydroxylase (F3'5'H) which is one of enzymes of *Commelina Communis* for synthesizing pigments.

BACKGROUND ART

[0002] The color of flower is a particularly important character in ornamental plants, and flowers having various colors have been produced by cross breeding heretofore. However, in the case of the cross breeding, gene sources are limited to species which are capable of cross breeding, and colors to be changed are limited. Further, in a case where only a specific character such as flower color is introduced into a specific variety, it is necessary to repeat backcrossing for long generations, and a lot of effort and time are required. Further, a period of cross breeding varies depending on plant species, and some plants take from a few years to a few decades for blossom. Particularly, Orchids such as moth orchid and cymbidium require a long time for blossom, and it takes a long time to develop such plants. Therefore, although demanded in markets, a superior new variety of moth orchid or cymbidium having a new flower color, particularly blue flower, has not been produced.

[0003] In recent years, it is possible to carry out cross breeding over species or genus by the recombinant DNA technology, and it is expected to produce a new variety having a color which cannot be obtained by the conventional cross breeding.

[0004] The color of flower derives mainly from three types of pigments: anthocyanin, carotenoid and betalain. Among them, anthocyanin (from orange to blue color) having the broadest maximum absorption wavelength has a role to govern blue color. Anthocyanin is one of flavonoids and biologically synthesized through a metabolic pathway shown in Fig. 1. The color of anthocyanin substantially depends on its chemical structure, and the more the number of hydroxyl groups in a benzene ring is, the more the color becomes blue. The hydroxylation of the benzene ring is catalyzed by a flavonoid 3'-hydroxylase (F3'H) and a flavonoid 3',5'-hydroxylase (F3'5'H). In a case where there is neither F3'H activity nor F3'5'H activity in petal cells, pelargonidin (from orange color to red color) is synthesized, and in a case where there is F3'H activity, cyanidin (from red to crimson color) is synthesized. Further, in a case where there is F3'5'H activity, delphinidin (blue color) is synthesized. Therefore, in order to produce the blue flower color, the role of F3'5'H is considered to be important.

[0005] From such a viewpoint, a study is in progress to produce a plant having a blue flower by the gene recombination using F3'5'H.

[0006] As conventionally known genes encoding the F3'5'H, genes derived from plants such as *Campanula medium, Catharanthus roseus, Petunia, Eustoma grandiflorum, Nierembergia sp., Verbena, Gentiana, Gossypium hirsutum, Lycianthes rantonnei, Solanum tuberosum* and *Torenia*, have been known, however, a gene encoding the F3'5'H which is isolated from a *Commelina communis* has not been reported.

[0007] As examples wherein a flower color is changed by using a conventionally known gene, a method for producing a blue carnation by transfecting a carnation DFR (dihydroflavonol 4-reductase)deficient variety with a F3'5'H gene and DFR gene which are derived from *Petunia* (Patent Document 1) and a method for producing a blue rose by transfecting a rose of which internal metabolism pathway is suppressed, with a F3'5'H gene derived from *Viola x wittrockiana* (Patent Document 2) have been reported.

[0008] On the other hand, it has been reported to change the flower color of moth orchid by overexpressing an endogenous gene, however, a blue moth orchid has not been produced (Non-Patent Document 1). Further, it has not been reported to have produced a blue variety of cymbidium.

Patent Document 1: WO1996/03671
Patent Document 2: WO2005/017147
Non-Patent Document 1: Su and Hsu, Biotechnology Letters (2003) 25: 1933-1939.

DISCLOSURE OF THE INVENTION

OBJECT TO BE ACCOMPLISHED BY THE INVENTION

[0009] In order to produce a blue flower, the F3'5'H is known to play an important role, and a F3'5'H gene which can be expressed more strongly has been desired.

[0010] It is an object of the present invention to find out a highly expressed type F3'5'H gene, whereby blue moth orchid can be produced, and to produce Orchids having a blue flower by using such a highly expressed type F3'5'H gene.

MEANS TO ACCOMPLISH THE OBJECT

[0011] The present inventors have conducted an extensive study in order to accomplish the above object, and as a result they have found that a F3'5'H gene which is derived from *Commelina communis* has a higher effect than that of conventional genes, and that by transfecting moth orchid with the above gene, the color of its flower can be changed to blue. Thus, the present invention has been accomplished.

[0012] That is, the present invention relates to a gene encoding a flavonoid 3',5'-hydroxylase of *Commelina communis*, which comprises an amino acid sequence depicted in SEQ ID No: 2 or an amino acid sequence having at least 90% of homology to the amino acid sequence depicted in SEQ ID No: 2.

[0013] Further, the present invention relates to a vector, which contains the above gene.

[0014] Further, the present invention relates to a method for producing a flower color-changed plant, which comprises transfecting a plant with the above gene and expressing the gene.

[0015] Further, the present invention relates to a method for producing a flower color-changed plant, which comprises transfecting an Orchid with the above gene and a gene encoding a dihydroflavonol 4-reductase of *Torenia* or *Gerbera* and expressing the genes.

[0016] Further, the present invention relates to a method for producing an Orchid having a blue flower, which comprises transfecting an Orchid having a white flower with the above gene; a gene encoding a dihydroflavonol 4-reductase of *Torenia* or *Gerbera;* a gene encoding a flavanone 3-hydroxylase; and a gene encoding an anthocyanidin synthase and expressing the genes.

[0017] Further, the present invention relates to a flower color-changed plant, which is produced by the above method, a progeny having the same characters as the changed flower color; or its tissue.

[0018] Further, in the present invention, "flavonoid 3',5'-hydroxylase" (F3'5'H) is an enzyme which catalyzes a reaction to produce dihydromyricetin from dihydrokaempferol. Further, "flavanone 3-hydroxylase" (F3H) is an enzyme which catalyzes a reaction to produce dihydrokaempferol from naringenin. "Dihydroflavonol 4-reductase" (DFR) is an enzyme which catalyzes a reaction to produce leucodelphinidin from dihydromyricetin. "Anthocyanidin Synthase" (ANS) is an enzyme which catalyzes a reaction to produce delphinidin from leucodelphinidin.

EFFECT OF THE PRESENT INVENTION

[0019] By using the F3'5'H gene derived from *Commelina communis*, plants having various blue flower colors can be produced. Particularly, by the present invention, it is possible to produce Orchids having blue flower colors which cannot be accomplished by the conventional cross breeding methods.

BRIEF DESCRIPTION OF DRAWINGS

[0020]

Fig. 1 is a synthetic pathway of anthocyanin.
Fig. 2 is a plasmid vector used for transfecting a petal with a gene. P-CaMV35S is a cauliflower mosaic virus 35S promoter. Ω is an omega sequence of tobacco mosaic virus. T is a transcription termination sequence derived from a cauliflower mosaic virus.
Fig. 3 is transfection of a petal of *Phal. amabilis* with a different ANS gene derived from a plant.
Fig. 4 is transfection of a petal of *Phal. amabilis* with a different DFR gene derived from a plant.
Fig. 5 is transfection of a petal of *Phal. amabilis* with a different F3'5'H gene derived from a plant.
Fig. 6 is an identification of a gene required for changing the flower color of a white moth orchid.
Fig. 7 is a schematic view (1) showing a procedure for construction of DNA for transformation of moth orchid.
Fig. 8 is a schematic view (2) of a procedure for construction of DNA for transformation of moth orchid.
Fig. 9 is transfection of a petal of a white cymbidium with genes.

BEST MODES FOR CARRYING OUT THE INVENTION

[0021] Now, the present invention will be described in detail, and by these descriptions, other objects, characteristics and superiorities of the present invention will become apparent for those skills in the art. Further, the descriptions of the present specification including the following explanations, specific examples, etc. show preferred embodiments, and those skilled in the art can make various changes and/or modifications (or alternations) of the invention within the concept

and the scope of the present invention disclosed in the present specification. Further, all Patent Documents and references cited in the present specification are cited for the purpose of explanation, and it should be understood that their contents are included as a part of the present specification.

[0022] By paying attention to a F3'5'H gene which relates to a blue pigment (delphinidin), the present inventors have transfected moth orchid with a known F3'5'H gene which is derived from petunia and has shown practical results in carnation, in order to produce blue orchids. However, the desired effect cannot be obtained. Thus, the present inventors have carried out cloning of F3'5'H genes of various plants in order to find a superior gene. As a result, they have found that a gene (sequence number: 1) derived from *Commelina communis* has a remarkably high pigment production performance. In a case where a petal cell of moth orchid is transfected with the above gene, five times of delphinidin is produced, as compared to the gene derived from petunia which is the prior art. Further, in a case where a petal cell of a red moth orchid is transfected with the gene of the present invention, the flower color is changed to blue tone purple.

[0023] Further, it has been found that the flower color is changed from white to blue by transfecting a petal cell of a white moth orchid with the F3H gene which catalyzes an up stream reaction than the F3'5'H and a DFR gene and an ANS gene which catalyze a down stream reaction than the F3'5'H in the synthesis pathway of anthocyanin, in addition to the above F3'5'H gene. Particularly, in a case where a DFR gene derived from *Gerbera* or *Torenia* is used, a flower has a deeper blue color.

[0024] The gene derived from *Commelina communis* which is found in the present invention is superior to conventional genes, and by employing the gene of present invention, it is possible to produce a blue type moth orchid. Further, the superiority of the gene of the present invention has been proven on cymbidium as well as on moth orchid, and it has been found that the gene of the present invention can be applied for changing flower colors of various flowering plants.

[0025] The F3'5'H gene is isolated from the genome DNA of Asiatic dayflower (nomenclature: *Commelina communis*) or cDNA reversetranscribed from a mRNA transcribed from the above genome DNA. The F3'5'H gene of the present invention is a gene depicted in SEQ ID NO.: 1 encoding a protein depicted in SEQ ID NO.: 2. The gene of the present invention includes genes having a high homology to the above gene, so long as such genes have the F3'5'H enzyme activity. The high homology may be at least 85% homology to the amino acid sequence depicted in SEQ ID NO.: 2, preferably at least 90%, more preferably at least 95%, further preferably at least 98%.

[0026] As the flavanone 3-hydroxylase (F3H) gene, the dihydroflavonol 4-reductase (DFR) gene and the anthocyanidin synthetic enzyme (ANS) gene, novel or conventional genes may be used. Specifically, the F3H gene may, for example, be a gene registered on GenBank (accession number: DQ394303, AY221246, AJ493133, AY641730, AF184270, AB078956, AB201760, AY669324, AF036093, AB211958, AB187027 or AB234905). The DFR gene may, for example, be a gene registered on GenBank (accession number: AAB62873, AAC17843, AAD49343, AAQ83576, AAU93766, AAY32600, AAY32601, AAY32602, BAB40789 or BAE79202). The ANS gene may, for example, be a gene registered on GenBank (accession number: AY585677, AY228485, AF015885, AY581048, U82432, AY695817, AB208689, AY997840, AY382828, AY256380, AF026058, Y07955, AF384050, AB097216, AB087206, AB198869, AB044091, AY123768 or AB234906). The DFR gene is particularly preferably a gene derived from *Gerbera* which is shown by accession number: Z17221 or a gene derived from *Torenia* which is shown by accession number: AB012924. Further, a novel gene which is found in the present invention such as the F3H gene (SEQ ID No: 50) which is derived from moth orchid, the DFR gene (SEQ ID No: 64) or the ANS gene (SEQ ID No: 74) may be used.

[0027] Further, the present invention relates to a recombinant vector containing such a gene, particularly an expression vector.

[0028] Depending on the host species into which the gene is introduced, the expression vector has an expression regulation region such as a promoter, a terminator, a DNA replication origin, etc. The promoter may, for example, be one which induces a gene expression in petal cells, and a promoter derived from a CHS (chalcone synthase) gene of moth orchid or a promoter derived from cauliflower mosaic virus (CaMV) 35S may be mentioned. Further, the terminator may, for example, be a terminator derived from a CHS gene of moth orchid or a terminator derived from cauliflower mosaic virus (CaMV) 35S.

[0029] Specifically, it is preferred that the F3'5'H gene of *Commelina communis* is connected to a 3' downstream of the promoter sequence, and a transcription termination sequence is added to a 3' downstream of the F3'5'H gene.

[0030] The F3H gene, the DFR gene and/or the ANS gene can also be connected to a 3' downstream of a promoter sequence and introduced into plants, like the F3'5'H gene.

[0031] The expression vector can be produced in accordance with a conventional method by using a restriction endonuclease, a ligase, etc. Further, transformation of hosts with the expression vector can also be carried out in accordance with a conventional method.

[0032] In the present invention, the method for expressing a gene in petal cells may, for example, be a microprojectile bombardment method, Agrobacterium-mediated transformation method, electroporation method, PEG method or virus-mediated transformation method.

[0033] Fig. 7 (pBIH-35S-CcF3'5'H) shows one example of a vector for carrying out transformation with the F3'5'H gene by the Agrobacterium-mediated transformation method. Further, the F3H, DFR and ANS genes can also be con-

structed on the same vector as containing the *Commelina communis* F3'5'H gene independently or together (Figs. 7 and 8).

[0034] In the present invention, plants to be transformed are flowering plants, preferably Orchids. The Orchids may, for example, be moth orchid, cymbidium, dendrobyobium, dendrochilum, oncidium, odontoglossum, mitonia or cattleya. Among them, moth orchid and cymbidium are preferred. Cymbidium is a plant which belongs to genus *Cymbidium,* and moth orchid includes genus *Phalaenopsis* and genus *Doritaenopsis.*

EXAMPLES

[0035] Now, the present invention will be described in further detail with reference to Examples. However these Examples are simply given to describe the present invention and as references of embodiments. These examples are to describe specific embodiments of the present invention and are not to restrict or limit the scope of the present invention. Further, it should be understood that various modes based on the technical concept of the present specification may be possible.

[0036] Further, general methods required for recombination of gene such as cutting and connecting DNA, transformation of *E. coli*, base sequence determination of a gene and PCR were basically carried out in accordance with manuals of commercially available reagents or apparatus used for each operation or experimental manual (such as "Molecular Cloning: A Laboratory Manual (Third Edition, Sambrook and Russell, 2001, Cold Spring Harbor Laboratory Press"). For PCR, GeneAmp PCR system 9700 (PE Applied Biosystems) was used. Unless otherwise specified, an apparatus was operated in accordance with a standard operation method described in a manual attached to the apparatus. Unless otherwise specified, all examples were carried out or may be carried out by using standard techniques, and such techniques are well known and common to those skills in the art.

[0037] As a homology searching program for examining an amino acid sequence encoded with the novel gene of the present invention and known amino acid sequences, BLASTP 2.2.15 (http://www.ddbj.nig.ac.jp/search/blast-j.html, Document: Altschul et al., Nucleic Acids Res. (1997) 25: 3389-3402) was used. Here, homology is the degree of similarity of amino acids over the entire sequence, and its value is obtained by aligning an amino acid sequence encoded with the novel gene and known amino acid sequences in order of the high similarity, and dividing the number of the homologous amino acids between them by the number of amino acids of the compared region. Here, high similarity is an alignment result output under a state (with gap, expected value 10, with filter) that the above BLASTP program parameter was defaulted. The homology described in the present Examples is one described with respect to a known amino acid sequence which showed the highest homology in the above homology analysis.

EXAMPLE 1: Transfecting a petal of moth orchid with a gene

[0038] Unless otherwise specified in Examples of the present specification, a petal of moth orchid was transfected with each gene by the following gene transfection method, and its property was evaluated. All genes had a DNA structure having a promoter at 5' side and a terminator at 3' side and were introduced into a petal cell in the form so as to express in the cell.

[0039] A bud of moth orchid was sterilized with a 1 wt% of sodium hypochlorite aqueous solution for five minutes and washed with sterilized water 3 times. Then, the bud was resolved into a lateral sepal, dorsal sepal and petal, and the lateral sepal, dorsal sepal and petal were left on an agar medium containing New Dogashima Medium salt (Tokuhara and Mii, Plant Cell Reports (1993) 13: 7-11., hereinafter referred to as NDM salt) and 0.6 wt% of agarose. Here, in the case of *Phal. amabilis*, a bud having a length of about 15 mm was used, and in the case of *Dtps. Queen Beer 'Mantenkou'*, a bud having a length of about 8 mm was used.

[0040] DNA to be introduced was purified by using Hi Speed Plasmid Midi Kit (QIAGEN), and the gene was introduced by the microprojectile bombardment method. Further, in a case where plural genes were introduced simultaneously, these DNA solutions were equally mixed one another, and such a mixture was used as a DNA solution for transfection.

[0041] At that time, the adsorption of DNA on gold particles was carried out at the following ratio. DNA dissolved in 20 μl of Tris/EDTA buffer (10 mM Tris-HCL, 1 mM EDTA, pH8.0) (Each 2 μg DNA of plasmid containing a gene was mixed and dissolved in the Tris/EDTA buffer.) was mixed with 50 μl of a gold particles suspension (the particle size: 1.0 μm, 60 mg/ml of 50% glycerol), and 50 μl of 2.5 M calcium chloride and 10 μl of 0.2 M spermidine were added to 70 μl of the mixture and suspended, whereby DNA was adsorbed on gold particles. Then, a supernatant was removed by centrifugal separation, and the particles were washed with 70% ethanol and 100% ethanol respectively. Then, 60 μl of 100% of ethanol was added to an obtained precipitate to prepare a suspension. The suspension was used as a sample solution, and 10 μl of the sample solution was used for one time of gene transfection. As a gene gun, IDERA GIE-III (TANAKA Co., Ltd.) was used. The gene transfection was carried out under a condition of the distance from a nozzle to a sample of 12 cm, under a reduced pressure of -80 kPa, a helium gas pressure of 0.3 MPa and spraying time of 0.025 second.

[0042] The petal after the gene transfection was left on an NDM salt agar medium and cultured under a light-dark cycle (light intensity: 23 μmol/m^2/s, light period: 16 hours, dark period: 8 hours) at 25°C.

EXAMPLE 2: Searching and cloning *Commelina communis* F3'5'H gene (CcF3'5'H)

[0043] From a petal of a bud of the blue colored Asiatic dayflower (*Commelina communis*), all RNA was extracted by using RNeasy Plant Mini Kit (QIAGEN), and by using the RNA as a template, cDNA was prepared by using GeneRacer kit (Invitrogen).

[0044] Then, RT-PCR was carried out by using this cDNA as a template. Primers used for the PCR reaction were 35FH-1 (5'-ATGGTIGTIGARYTIATGAC-3'; SEQ ID No.: 3) and 35FH-4 (5'-CCRAAIGGIATIARYTCRAA-3'; SEQ ID NO.: 4) which were designed from a sequence of a conventionally known F3'5'H gene (GenBank accession No.: D14590, AJ011862, AB262585, D14589, AB078514, AY566988, D85184, AY275430, AF313490, AY675558, AB012925). In the reaction, a step of 98°C for 10 seconds, 56°C for 30 seconds and 72°C for 1 minute was repeated 40 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template and a primer 35FH-2 (5'-TGGATG-GAYYTICARGGIAT-3'; SEQ ID NO.: 5) and a primer 35FH-3(5'-CCDATIGCCCADATRTTIAC-3'; SEQ ID NO.: 6). In the reaction, a step of 98°C for 10 seconds, 56°C for 30 seconds and 72°C for 1 minute was repeated 40 cycles. An obtained reaction product was subjected to terminal blunting treatment and then cloning to HincII site of pUC18 (Takara Bio Inc.) to obtain p35FH23, and a partial DNA sequence contained in the p35FH23 was determined (CcF3'5'H partial sequence).

[0045] From the CcF3'5'H partial sequence, sequences of the 3' downstream side and 5' upstream side were analyzed by the RACE method.

[0046] The 3'RACE method was carried out by using a primer which can be designed from the CcF3'5'H partial sequence, the above *Commelina communis* RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were C35FH-3 (5'-ATCTCCCTCGTATCGCAACC-3'; SEQ ID NO.: 7) and GeneRacer 3' primer. In the reaction, a step of 98°C for 10 seconds, 58°C for 30 seconds and 72°C for one minute was repeated 40 cycles. Nested PCR was carried out by using an obtained reaction solution as a template, a primer C35FH-4 (5'-GAAGCTTGTGAAGCCAATGG-3'; SEQ ID NO.: 8) and GeneRacer 3' Nested primer. In the reaction, a step of 98°C for 10 seconds, 58°C for 30 seconds and 72°C for 1 minute was repeated 40 cycles. An obtained reaction product was subjected to termination blunting treatment and then cloning to HincII site of pUC18 (Takara Bio Inc.) to obtain p35FHC43', and a DNA sequence of a 3' downstream side contained in the p35FHC43' was determined (CcF3'5'H 3'RACE sequence).

[0047] The 5'RACE method was carried out by using a primer which can be designed from the CcF3'5'H partial sequence, the above *Commelina communis* RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were C35FH-6 (5'-CGTCGCCTCGTGCTCTCGCAGTATC-3'; SEQ ID NO.: 9) and GeneRacer 5' primer. In the reaction, a step of 98°C for 10 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 30 cycles. Nested PCR was carried out by using an obtained reaction solution as a template, a primer C35FH-5 (5'-TCTTCGAGAGCACCTTATC-GAACCTC-3'; SEQ ID NO.: 10) and GeneRacer 5' Nested primer. In the reaction, a step of 98°C for 10 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 30 cycles. An obtained reaction products was subjected to cloning to pCR4/TOPO10 (Invitrogen) to obtain p35FH5'5, and a DNA sequence of a 5' upstream side contained in the p35FH5'5 was determined (CcF3'5'H 5'RACE sequence).

[0048] The entire *Commelina communis* F3'5'H gene (CcF3'5'H) was subjected to cloning based on the CcF3'5'H 3'RACE sequence and the CcF3'5'H 5'RACE sequence. RT-PCR was carried out by using the above *Commelina communis* RNA as a template, a primer C35FH-7(5'-GAAAACCAATACAAAAACATACC-3'; SEQ ID NO.: 11), a primer C35FH-10 (5'-ATTGCTTCAAGTTCCCTAGC-3'; SEQ ID NO.: 12) and Ready-To-Go RT-PCR Beads (Amersham Bio-sciences). In the reaction, one step of 94°C for 30 seconds, 54°C for 30 seconds and 72°C for 2 minutes was repeated 30 cycles. Further, semi Nested PCR was carried out by using an obtained reaction solution as a template, a primer C35FH-7 (SEQ ID NO.: 11) and C35FH-9 (5'-GTTCCCTAGCCCCGTACCAC-3'; SEQ ID NO.: 13). In the reaction, a step of 98°C for 10 seconds, 54°C for 30 seconds and 72°C for 1 minute was repeated 30 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO10 (Invitrogen) to obtain p35FH79. Then, a DNA sequence of the entire Asiatic dayflower F3'5'H gene contained in the p35FH79 was determined (CcF3'5'H; SEQ ID NO.: 1). Further, the sequence of the gene of the present invention found in *Commelina communis* is a novel gene. The amino acid sequence encoded by the base sequence has 6.1 % homology to the amino acid sequence (GenBank accession No.: AY856345) encoded by the F3'5'H gene of delphinium by the homology analysis.

EXAMPLE 3: Preparation of an expression vector (pBS-P35T35) for gene transfection

[0049] pBS-P35T35 is a plasmid having a cauliflower mosaic virus 35S promoter (Hohn et al., Curent Topics in Microbiology and Immunology (1982) 96: 194-236), an omega sequence of tobacco mosaic virus (Gallie et al., Nucleic Acids Research (1987) 15: 3257-3273), a restriction endonuclease SwaI site and a cauliflower mosaic virus 35S terminator

in this order in pBluescriptIISK-(Stratagene) (Fig. 2). A plasmid having the substantially same function as this pBS-P35T35 can be constructed as follows.

[0050] An oligonucleotide SAS-S (5'-CTAGCTAGCGGCGCGCCTGCAGGATATCATTTAAATCCCGGG-3'; SEQ ID NO.: 14) and an oligonucleotide SAS-AS (5'-CCCGGGATTTAAATGATATCCTGCAGGCGCGCCGCTAGCTAG-3'; SEQ ID NO.: 15) were denatured and then gradually cooled to room temperature. An obtained one was subjected to Nhel treatment and connected to Xbal-EcoRV site of pBluescriptIISK-(Stratagene) to prepare pBS-SAS which is a plasmid DNA of which a restriction enzyme site is modified. A region amplified by PCR using a cauliflower mosaic virus genome DNA (GenBank accession V00140) as a template, a primer T-CaMV35S-SseI-F (5'-AACCTGCAGGAAATCAC-CAGTCTCTCTCTA-3'; SEQ ID NO.: 16) and a primer T-CaMV35S-AscI-R (5'-GGCGCGCCATCGATAAGGGGTTATT-AG-3'; SEQ ID NO.: 17) was treated with a restriction endonuclease Sse83871 and AscI. 1. This fragment was connected to Sse83871-AscI site of pBS-SAS to prepare pBS-T35S. Based on pJD301 (Leon et al., Plant Physiology (1991) 95: 968-972), a sequence of cauliflower mosaic virus 35S promoter which is cut by HindIII and HincII and an omega sequence of tobacco mosaic virus were connected to HindIII-SmaI site of pBS-T35S to prepare an expression vector (pBS-P35T35).

EXAMPLE 4: Subcloning of the *Commelina communis* F3'5'H gene to an expression vector

[0051] An Open Reading Frame part of the *Commelina communis* F3'5'H gene was amplified by PCR using a plasmid DNA (p35FH79) containing the above CcF3'5'H entire sequence as a template and was subjected to subcloning to an expression vector pBS-P35T35. The PCR was carried out by using a primer CcF35H-F (5'-ATGGTACCCCTTACGTAC-CTT-3'; SEQ ID NO.: 18), a primer CcF35H-R (5'-TTATGTTGTTTTTATATTCTTATAAACG-3'; SEQ ID NO.: 19) and p35FH79 as a template. In the reaction, a step of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 minute and 20 seconds was repeated 25 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain p35CcF3'5'H. The p35CcF3'5'H is DNA to express the *Commelina communis* F3'5'H gene in plant cells.

EXAMPLE 5: Confirmation of expression of the *Commelina communis* F3'5'H gene

[0052] A petal of a red moth orchid (*Dtps. Queen Beer 'Mantenkou'*) was transfected with 1.2 μg of the p35CcF3'5'H by the method of Example 1 and cultured for 5 days, whereby many deep purple cells emerged at the petal. On the other hand, in a case where cells were transfected with gold particles containing no such a gene, the above phenomena was not observed.

[0053] Thus, it is considered that the isolated *Commelina communis* F3'5'H gene imparted a flavonoid 3'5'-hydroxylase activity to the petal cell of the moth orchid, and as a result, delphinidin which is a blue pigment was produced.

EXAMPLE 6: Evaluation of enzyme activity of the *Commelina communis* F3'5'H gene

[0054] The enzyme activity of the *Commelina communis* F3'5'H gene was evaluated by the amount of delphinidin which is a blue pigment and compared to an already known gene (Petunia gene) which is considered to be useful for producing blue carnation.

(1) Isolation of the Petunia F3'5'H gene (PetF3'5'H)

[0055] All RNA was isolated from a petal of a bud of commercially available Petunia (hybrid) before blossom by using RNeasy Plant Mini Kit (QIAGEN). cDNA was prepared by using this RNA as a template and superscriptII First-Strand Synthesis System (Invitrogen). Then, RT-PCR was carried out by using this cDNA as a template. As primers for the PCR reaction, PetF3'5'H1-F (5'-ATGATGCTACTTACTGAGCTTGGTG-3'; SEQ ID NO.: 20) and PetF3'5'H1-R (5'-CAA-CATGCGCAATTATAGCA-3'; SEQ ID NO.: 22) which were designed from two sequences (GenBank accession No.: A29011, A29013) of an already known petunia F3'5'H gene, or PetF3'5'H2-F(5'-ATGGTGCTACTTAGTGAGCTTGC-3'; SEQ ID NO.: 22) and PetF3'5'H2-R (5'-AACCAACGTAAAGGCATGTT-3'; SEQ ID NO.: 23) were used. In both cases, the reaction was carried out by repeating a step of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 2.5 minutes 45 cycles. Each obtained reaction product was subjected to cloning at SwaI site of pBS-P35T35 to obtain p35PetF3'5'H1 and p35PetF3'5'H2.

(2) Transfection with *Commelina communis* F3'5'H gene and Petunia F3'5'H gene

[0056] A petal of a red moth *orchid* (*Dtps. Queen Beer 'Mantenkou'*) was transfected with 1.3 μg of either the p35CcF3'5'H gene derived from *Commelina communis* or a gene (p35PetF3'5'H1 or p35PetF3'5'H2) derived from Petunia by the method of Example 1. Further, at the time of gene transfection, in order to measure efficiency of gene transfection, the petal was cotransfected with 0.17 μg of p35luc (Firefly Luciferase gene) as the internal standard. This

p35luc was prepared by subcloning the blunt-ended Luciferase gene fragment, obtained by cutting pSP-luc+(Promega) with BglII-XbaI, into the blunt-ended pB1221 (CLONTECH) cut with BamHI and SacI.

[0057] The gene transfected petal was static cultured for 5 days, and then grinded in liquid nitrogen and suspended in 200 μl of 0.1×Passive Lysis Buffer (Dual-Luciferase Reporter Assay System, Promega) to prepare a sample. The Luciferase activity of the sample was measured, and the quantitative analysis of anthocyanidin was carried out.

MEASUREMENT OF THE LUCIFERASE ACTIVITY

[0058] 5 μl of 5xPassive Lysis Buffer (Promega) and 100 μl of Luciferase Assay Substrate (Promega) were added to 20 μl of the above sample, and then the Luciferase activity was measured by using luminometer flash'n glow LB955 (BERTHOLD TECHNOLOGIES) under a condition of measuring time of 10 seconds.

QUANTITATIVE ANALYSIS OF DELPHINIDIN

[0059] 400 μl of 2N hydrochloric acid was added to 150 μl of the above sample, and then hydrolysis treatment was carried out at 98°C for 2 hours, and extraction with 200 μl of isoamylalcohol was carried out. The amount of delphinidin in the organic layer was measured by a liquid chromatography method under the following condition.

Apparatus: Waters2690 (Waters),
Column: Nucleosil 100-5C18 4.6x250 mm (GL. Sciences),
Column temperature: 40°C,
Elution condition: Gradient (B liquid 20% → 85% for 40 minutes, B liquid 85% for 5 minutes) was applied by using 1.5% of phosphoric acid solution (A liquid) and 1.5% phosphoric acid 20% acetic acid 25% acetonitrile aqueous solution (B liquid), and elution was carried out at a flow rate of 1 ml /minute.
Detection wavelength: 531 nm.

[0060] The amount of delphinidin was measured by an absolute analytical curve method using the standard (delphinidin hydrochloride). Further, the obtained amount of delphinidin was corrected by using Luciferase activity. Results are shown in Table 1. It is evident from Table 1 that the accumulated amount of delphinidin by the *Commelina communis* F3'5'H gene was higher by about five times than that of the Petunia F3'5'H2 gene. Further, the delphinidin was not detected from the sample using the Petunia F3'5'H1 gene.

TABLE 1

	CcF3'5'H			PetF3'5'H2		
	Amount of delphinidin (pg/sample)	Luc assay (RLU/sample)	Pg/RLU	Amount of delphinidin (pg/sample)	Luc assay (RLU/sample)	pg/RLU
1	12400	265681	0.0467	4400	491562	0.0090
2	26200	743973	0.0352	13000	1063867	0.0122
3	50600	880990	0.0574	7400	757137	0.0098
4	37000	741444	0.0499	3200	530822	0.0060
Average			0.0473			0.0092
SD			0.0092			0.0026

EXAMPLE 7: Transfection of a petal of the white moth orchid with a F3'5'H gene

[0061] In order to produce a blue moth orchid, a petal of white moth orchid (*Phal. amabilis*) was transfected with 1.6 μg of either a gene (p35CcF3'5'H) derived from *Commelina communis* or a gene (p35PetF3'5'H1, p35PetF3'5'H2) derived from Petunia, like Example 6(2), in accordance with the method of Example 1. However, though the petal was transfected with such a gene, clear color change of the petal was not observed.

[0062] Therefore, a gene encoding the group of enzymes at the upstream of the F3'5'H in the anthocyanidin synthetic pathway (Fig. 1) ((1) Chalcone synthase: CHS, (2) Chalcone isomerase: CHI and (3) flavanone 3-hydroxylase: F3H) was isolated for transfection.

EXAMPLE 8: Isolation of the moth orchid CHS gene (PhCHS3)

[0063] All RNA was isolated from a petal just before blossom of moth orchid (*Dtps. Sogo Vivien*×*Dtps. Sogo Yenlin*) by using RNeasy Plant Mini Kit (QIAGEN). cDNA was prepared by using this RNA as a template and Superscriptll First-Strand Synthesis System (Invitrogen). Then, RT-PCR was carried out by using this cDNA as a template. As primers for the PCR reaction, PhCHS3 F1 (5'-AAGCTTGTGAGAGACGACGGA-3'; SEQ ID NO.: 24) and PhCHS3 R1 (5'-TGGCCCTAATCCTTCAAATT-3'; SEQ ID NO.: 25) which were designed from the known moth orchid CHS gene (PhCHS) sequence (GenBank accession No.: DQ089652) were used. The reaction was carried out by repeating a step of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute 25 cycles. A reaction product was amplified by using this reaction solution as a template under the same condition again. An obtained reaction product was subjected to cloning at SwaI site of pBS-P35T35 to obtain p35PhCHS3. Then, a DNA sequence of the entire moth orchid CHS gene contained in p35PhCHS3 was determined (PhCHS3; SEQ ID NO.: 26). The p35PhCHS3 is DNA for expressing the moth orchid CHS gene in plant cells.

EXAMPLE 9: Isolation of Moth orchid CHI gene (PhCHI1)

[0064] All RNA was isolated from a petal just before blossom of moth orchid (*Dtps. Sogo Vivien*×*Dtps. Sogo Yenlin*) by using RNeasy Plant Mini Kit (QIAGEN). cDNA was prepared by using this RNA as a template and Superscriptll First-Strand Synthesis System (Invitrogen).

[0065] Then, RT-PCR was carried out by using this cDNA as a template. Various plants' CHI genes have been reported (GenBank accession No.: AY700850, AY086088, DQ160231, AJ004902, AF474923, XM_470129, U03433, AB187026). As primers for the PCR reaction, CHI-dgF1 (5'-TTYCTCGSYGGBGCMGGYGWVMGVGG-3'; SEQ ID NO.: 28) and CHI-dgR1 (5'-CMGGIGAIACVSCRTKYTYICCRATVAT-3'; SEQ ID NO.: 29) which were designed from the conventionally known CHI gene were used. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles.

[0066] Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer CHI-dgF3 (5'-TMIKYWCMGGISMITTYGARAARYT-3'; SEQ ID NO.: 30) and a primer CHI-dgR3 (5'-TYICCRATVATIGWHTCCAR-IAYBGC -3'; SEQ ID NO.:31). In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning at pCR4-TOPO (Invitrogen) to obtain PhCHIfrag 16, and a partial DNA sequence contained in the PhCHIfrag 16 was determined (PhCHI partial sequence).

[0067] From the PhCHI partial sequence, sequences of the 3' downstream side and 5' upstream side were analyzed by the RACE method.

[0068] The 3'RACE method was carried out by using a primer which can be designed from the PhCHI partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhCHI-GSP F1 (5'-ATGCT-GCTGCCATTAACGGGTCA-3'; SEQ ID NO.: 32) and GeneRacer 3' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhCHI-GSP F2 (5'-TCCGAGAAGGTCTCCGGGAACT-3'; SEQ ID NO.: 33) and GeneRacer 3' Nested primer. In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4-TOPO (Invitrogen) to obtain PhCHI3'RACE23. A DNA sequence of the 3' downstream side contained in the PhCHI3'RACE23 were determined (PhCHI3'RACE sequence).

[0069] The 5'RACE method was carried out by using a primer which can be designed from the PhCHI partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhCHI-GSP R1 (5'-GCAT-TCGTCAGCTTCTTGCTCTCT-3'; SEQ ID NO.: 34) and GeneRacer 5' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhCHI-GSP R2 (5'-ATCACATCAGTCTCAGCCACA-3'; SEQ ID NO.: 35) and GeneRacer 5' Nested primer. In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4-TOPO (Invitrogen) to obtain PhCH15'RACE54. A DNA sequences of the 5' upstream side contained in the PhCH15'RACE54 was determined (PhCH15'RACE sequence).

[0070] The entire of the moth orchid CHI gene (PhCHI) was subjected to cloning based on the PhCHI3'RACE sequence and the PhCH15'RACE sequence. PCR was carried out by using the above cDNA, a primer PhCHI init (5'-ATGGCA-GAAACAGTGGCGACGCCCA-3'; SEQ ID NO.: 36) and a primer PhCHI term(5'-TCAAACGACTCCATCTTGCTC-3'; SEQ ID NO.: 37). In the reaction, a step of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1.5 minutes was repeated 45 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain

p35PhCHI1. Then, a base sequence of the entire moth orchid CHI gene contained in the p35PhCHI1 was determined (PhCHI1, SEQ ID NO.: 38). Further, the present sequence of the gene found in moth orchid is a novel gene. The amino acid sequence encoded by the DNA sequence has 54% homology to the amino acid sequence (GenBank accession No.: DQ120521) encoded by the CHI gene of tea plant by the homology analysis. The p35PhCHI1 is DNA for expressing the moth orchid CHI gene in plant cells.

EXAMPLE 10: Isolation of moth orchid F3H gene (PhF3H1)

[0071] All RNA was isolated from a petal just before blossom of moth orchid (*Dtps. Sogo Vivien*×*Dtps. Sogo Yenlin*) by using RNeasy Plant Mini Kit (QIAGEN). cDNA was prepared by using this RNA as a template and Superscriptll First-Strand Synthesis System (Invitrogen).

[0072] Then, RT-PCR was carried out by using this cDNA as a template. Various plants' F3H genes have been reported (GenBank accession No.: DQ394303, AY221246, AJ493133, AY641730, AF184270, AB078956, AB078956, AB201760, AY669324, AF036093, AB211958, AB187027, AB234905). As primers for the PCR reaction, a primer F3H-dgF1 (5'-TIVGIGAYGARGABGARMGBCCIAA-3'; SEQ ID NO.: 40) and a primer F3H-dgR1 (5'-ACBGCYYGRTGRTCHGCRT-TCTTRAA-3'; SEQ ID NO.: 41) which were designed from the conventionally known F3H gene were used. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds, and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer F3H-dgF3 (5'-AARYTBRGKTTYGAYATGWCHGGIG-3'; SEQ ID NO.: 42) and a primer F3H-dgR3 (5'-GGHWS-RACVGTDATCCAIGWBTT -3'; SEQ ID NO.:43). In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4-TOPO (Invitrogen) to obtain PhF3Hfrag 26, and a partial DNA sequence contained in the PhF3Hfrag 26 was determined (PhF3H partial sequence).

[0073] Sequences of the 3' downstream side and 5' upstream side from the PhF3H partial sequence were analyzed by the RACE method.

[0074] The 3'RACE method was carried out by using a primer which can be designed from the PhF3H partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhF3H -GSP F1 (5'-TTCTCATACCCAATCGGGAG-3'; SEQ ID NO.: 44) and GeneRacer 3' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, and a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhF3H-GSP F2 (5'-AATCGGGAGCCGCGATTACT-3'; SEQ ID NO.: 45) and GeneRacer 3' Nested primer. In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4-TOPO (Invitrogen) to obtain PhF3H3'RACE33. A DNA sequence of the 3' downstream side contained in the PhF3H3'RACE33 was determined (PhF3H 3'RACEsequence).

[0075] The 5'RACE method was carried out by using an oligonucleotide which can be designed from the PhF3H partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhF3H -GSPR1 (5'-TCTGTGTGGCGCTTCAGGCC-3'; SEQ ID NO.: 46) and GeneRacer 5' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, and a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution, PhF3H-GSP R2 (5'-TGAGGTC-CGGTTGCGGGCATTTT-3'; SEQ ID NO.: 47) and GeneRacer 5' Nested primer. In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4-TOPO (Invitrogen) to obtain PhF3H5'RACE86. A DNA sequence of the 5' upstream side contained in the PhF3H5'RACE86 was determined (PhF3H 5'RACEsequence).

[0076] The entire of the moth orchid F3H gene was subjected to cloning based on the PhF3H 3'RACE sequence and the PhF3H 5'RACE sequence. PCR was carried out by using the above cDNA, a primer PhF3H init. (5'-AT-GGCCCCAATACCATTCCTACCGA-3' ; SEQ ID NO.: 48) and a primer PhF3H term. (5'-CCTTAAGCTAAAATCTCATT-TAATGCCTTTGCTCC-3' ; SEQ ID NO.: 49).

[0077] In the reaction, a step of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1.5 minute was repeated 40 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain p35PhF3H1. Then, a DNA sequence of the entire moth orchid F3H gene contained in the p35PhF3H1 was determined (PhF3H1; SEQ ID NO.: 50). Further, the sequence of the gene found in moth orchid is a novel gene. The amino acid sequence encoded by the DNA sequence has 86% homology to the amino acid sequence (GenBank accession No.: X89199) encoded by the F3H gene of *Bromheadia finlaysoniana*. The p35PhF3H1 is DNA for expressing the moth orchid F3H gene in plant cells.

EXAMPLE 11: Transfection of a petal of white moth orchid with the F3'5'H gene and anthocyanin related genes

[0078] A petal of a white moth orchid (*Phal. amabilis*) was cotransfected with the CHS gene (p35PhCHS3: Example 8), CHI gene (p35PhCHI1: Example 9) and F3H gene (p35PhF3H1: Example 10) which were derived from moth orchid and the F3'5'H gene (p35CcF3'5'H: Example 2) which was derived from *Commelina communis* in accordance with the method of Example 1. However, the color change of the petal was not observed.

[0079] Therefore, genes encoding enzymes ((1) dihydroflavonol 4-reductase: DFR and (2) anthocyanidin synthase: ANS) at downstream of the F3'5'H gene in the anthocyanin synthetic pathway were isolated for transfection.

EXAMPLE 12: Isolation of moth orchid DFR gene (PhDFR)

[0080] All RNA was isolated from a petal in a bud of moth orchid (*Dtps. Queen Beer 'Mantenkou'*) by using RNeasy Plant Mini Kit (QIAGEN), and cDNA was prepared by using this RNA as a template and GeneRacer kit (Invitrogen).

[0081] Then, RT-PCR was carried out by using this cDNA as a template. Various plants' DFR genes have been reported (GenBank accession No.: AAB62873, AAC17843, AAD49343, AAQ83576, AAU93766, AAY32600, AAY32601, AAY32602, BAB40789, BAE79202). Primers used for the PCR reaction were DFRD-F1 (5'-TTYCAYGTIGCIACNCC-NATG-3'; SEQ ID NO.: 52) and DFRD-R1 (5'-DATNGCRTCRTCRAACATYTC-3'; SEQ ID NO.: 53) which were designed from the sequence of the above DFR gene. In the reaction, a step of 94°C for 30 seconds, 48°C for 30 seconds and 72°C for 1 minute was repeated 40 cycles. Nested PCR was carried out by using an obtained reaction solution as a template, a primer DFRD-F2 (5'-ATGAAYTTYCARWSIRARGAYCC-3'; SEQ ID NO.: 54) and a primer DFRD-R2 (5'-RCAIATRTAICKNCIRTTNGC-3'; SEQ ID NO.:55). In the reaction, a step of 94°C for 30 seconds, 48°C for 30 seconds and 72°C for 1 minute was repeated 40 cycles. Further, Nested PCR was carried out again by using an obtained reaction solution as a template, a primer DFRD-F3 (5'-GARAAYGARGTNATHAARCC-3'; SEQ ID NO.: 56) and a primer DFRD-R3 (5'-RTCRTCIARRTGNACRAAYTG-3'; SEQ ID NO.:57). In the reaction, a step of 94°C for 30 seconds, 48°C for 30 seconds and 72°C for 30 seconds was repeated 40 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO (Invitrogen) to obtain PhDFR-D/pCR4, and a partial DNA sequence contained in the PhDFR-D/pCR4 was determined (PhDFR partial sequence).

[0082] From the PhDFR partial sequence, sequences of the 3' downstream side and 5' upstream side were analyzed by the RACE method.

[0083] The 3'RACE method was carried out by using a primer which can be designed from the PhDFR partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used for the PCR reaction were PhDFR -F1 (5'-GGTCAT-GCAAAAGGTCGGGCAGCGTAA-3'; SEQ ID NO.: 58) and GeneRacer 3' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhDFR-F2 (5'-GTGATCTTCACATCTTCCGCAGGAACAGT-3'; SEQ ID NO.: 59) and GeneRacer 3' Nested primer. In the reaction, a step of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO10 (Invitrogen) to obtain PhDFR3'RACE/pCR4, and a DNA sequence of 3' downstream side contained in the PhDFR3'RACE/pCR4 was determined (PhDFR3'RACE sequence).

[0084] The 5'RACE method was carried out by using a primer which can be designed from the PhDFR partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhDFR -R4 (5'-ATGATTCAT-TAAAAATCCGAAAAAAAGACCACTACAA-3'; SEQ ID NO.: 60) and GeneRacer 5' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1 minute was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhDFR-R3 (5'-AACCATGCATAATAAAGCAGATGTGTAAAT-3'; SEQ ID NO.: 61) and GeneRacer 5' Nested primer. In the reaction, a step of 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1 minute was repeated 25 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO10 (Invitrogen) to obtain PhDFR 5'RACE/pCR4, and an up-stream side DNA sequence contained in the PhDFR 5'RACE/pCR4 was determined (PhDFR 5'RACE sequence).

[0085] The entire moth orchid DFR gene was subjected to cloning based on the PhDFR3'RACE sequence and the PhDFR 5'RACE sequence. RT-PCR was carried out by using the above RNA , a primer PhDFR-F8A5 (5'-AAAAAAT-GGAGGATGTGAGGAAGGGTCCTGTT-3'; SEQ ID NO.: 62), a primer PhDFR-R5 (5'-ACATGATTCATTAAAAATC-CGAAAAAAAGACCA-3'; SEQ ID NO.: 63) and Ready-To-Go You Prime First Strand Beads (Amersham Biosciences). In the reaction, a step of 98°C for 30 seconds, 68°C for 30 seconds and 72°C for 1.5 minutes was repeated 35 cycles. An obtained reaction product was subjected to cloning to pBS-P35T35 to obtain p35PhDFR. Then, the entire DNA sequence of the DFR gene contained in the p35PhDFR was determined (PhDFR; SEQ ID NO.: 64). Further, the sequence of the gene found in moth orchid is a novel gene. The amino acid sequence encoded by the DNA sequence has 86% homology to the amino acid sequence (GenBank accession No.: AF007096) encoded by the DFR gene of *Bromheadia*

finlaysoniana by the homology analysis. The p35PhDFR is DNA for expressing the moth orchid DFR gene in plant cells.

EXAMPLE 13: Isolation of moth orchid ANS gene (PhANS1)

[0086] All RNA was isolated from a petal in a bud just before blossom of moth orchid (*Dtps. Queen Beer'Mantenkou*) by using RNeasy Plant Mini Kit (OIAGEN), and cDNA was prepared by using this RNA as a template and Superscriptll First-Strand Synthesis System (Invitrogen).

[0087] Then, RT-PCR was carried out by using this cDNA as a template. Primers used for the PCR reaction were ANS-dgF2 (5'-TICARGGBTAYGGIAGYARRYTIGCIRMYA-3'; SEQ ID NO.: 66) and ANS-dgR2 (5'-GGYT-CRCARAAIAYIRCCCAIGADA-3'; SEQ ID NO.: 67) which were designed from a known ANS gene. In the reaction, a step of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1.5 minutes was repeated 40 cycles. By using an obtained reaction solution as a template and the same primers again, a step of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute was repeated 30 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO10 (Invitrogen) to obtain PhANSfrag10, and a partial DNA sequence contained in the PhANSfrag10 was determined (PhANS partial sequence). From the PhANS partial sequence, sequences of the 3' downstream side and 5' upstream side were analyzed by the RACE method.

[0088] The 3'RACE method was carried out by using a primer which can be designed from the PhANS partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used for the PCR reaction were PhANS3RACEGSP1 (5'-GCCCACACCGACGTCAGCTCCCTCTCCT-3'; SEQ ID NO.: 68) and GeneRacer 3' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1.5 minutes was repeated 5 cycles, a step of 94°C for 30 seconds and 70°C for 1.5 minutes was repeated 5 cycles, and then a step of 94°C for 30 seconds, 70°C for 30 seconds and 72°C for 1.5 minutes was repeated 25 cycles. Further, Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhANS3RACEGSP2 (5'-CGTCGGGGATGCGCTCGAGATCCTCAGC-3'; SEQ ID NO.: 69) and GeneRacer 3' Nested primer. In the reaction, a step of 94°C for 10 seconds, 58°C for 10 seconds and 72°C for 1 minute was repeated 35 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO (Invitrogen) to obtain PhANS3'RACE37, and a DNA sequence of 3' down stream side contained in the PhANS3'RACE37was determined (PhANS3'RACE sequence).

[0089] The 5'RACE method was carried out by using a primer which can be designed from the PhANS partial sequence, the above RNA and GeneRacer kit (Invitrogen). The primers used in the PCR reaction were PhANS5RACEGSP1 (5'-AGTCCGCGGGTTCAGTCGGCCAGATGGT-3'; SEQ ID NO.: 70) and GeneRacer 5' primer. In the reaction, a step of 94°C for 30 seconds and 72°C for 1.5 minutes was repeated 5 cycles, a step. of 94°C for 30 seconds and 70°C for 1.5 minute was repeated 5 cycles, and then a step of 94°C for 30 seconds, 70°C for 30 seconds and 72°C for 1.5 minutes was repeated 25 cycles. Nested PCR was carried out by using an obtained reaction solution as a template, a primer PhANS5RACEGSP2 (5'-CCGTCTTCTCCGGCGGGTAGACGAGGTG-3'; SEQ ID NO.: 71) and GeneRacer 5' Nested primer. In the reaction, a step of 94°C for 10 seconds, 58°C for 10 seconds and 72°C for 1 minute was repeated 35 cycles. An obtained reaction product was subjected to cloning to pCR4/TOPO (Invitrogen) to obtain PhANS5'RACE15, and an upstream side DNA sequence contained in the PhANS5'RACE15 was determined (PhANS 5'RACE sequence).

[0090] The entire moth orchid ANS gene was subjected to cloning based on the PhANS 3'RACE sequence and the PhANS 5'RACE sequence. PCR was carried out by using the above cDNA, a primer PhANS init (5'-ATGGCCACCAAAG-CAATCCCACC-3'; SEQ ID NO.: 72), and a primer PhANS term (5'-TCAATCCACAGGCGCCTTCT-3'; SEQ ID NO.: 73). In the reaction, a step of 94°C for 30 seconds, 69°C for 30 seconds and 72°C for 1.5 minutes was repeated 35 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain p35PhANS1. Then, the entire base sequence of the ANS gene (PhANS1) contained in the p35PhANS1 was determined (PhANS1; SEQ ID NO.: 74). Further, the sequence of the present gene found in moth orchid is a novel gene. The amino acid sequence encoded by the DNA sequence has 58% homology to the amino acid sequence (GenBank accession No.: EF079869) encoded by the ANS gene of Anthurium by the homology analysis. The p35PhANS1 is DNA for expressing the moth orchid ANS gene in plant cells.

EXAMPLE 14: Transfection of a petal of white moth orchid with the F3'5'H gene and an anthocyanidin related gene

[0091] A petal of a white moth orchid (*Phal. amabilis*) was cotransfected with the F3'5'H gene which was derived from *Commelina communis* and the CHS (p35PhCHS3), CHI (35PhCHI), F3H (p35PhF3H1), DFR (PhDFR) and AnS (PhANS1) genes which were derived from moth orchid in accordance with the method of Example 1. As a result, light blue cells were observed at the petal.

[0092] Therefore, it is evident that in order to change flower color of a white moth orchid to blue, the DFR gene and ANS gene are important. Thus, in order to change flower color to deeper blue, other plants' DFR genes and ANS genes were studied.

EXAMPLE 15: Isolation and the DFR gene and ANS gene Gerbera and cotransfection therewith

(1) Isolation of the Gerbera DFR gene (GerDFR)

[0093] All RNA was isolated from a petal of a bud of commercially available Gerbera (hybrid) by using RNeasy Plant Mini Kit (QIAGEN), and cDNA was prepared by using this RNA as a template and Superscriptll First-Strand Synthesis System (Invitrogen). Then, RT-PCR was carried out by using this cDNA as a template. As primers for the PCR reaction, GerDFR-F (5'-ATGGAAGAGGATTCTCCGGC-3'; SEQ ID NO.: 76) and GerDFR-R (5'-CTATTGGCCTTCTTTT-GAACAACAAA-3'; SEQ ID NO.: 77) which were designed from a sequence (GenBank accession No.: Z17221) of a known Gerbera DFR gene (GerDFR) were used. The reaction was carried out by repeating a step of 98°C for 10 seconds, 55°C for 10 seconds and 72°C for 1 minute and 30 sconds 45 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain a Gerbera DFR gene (p35GerDFR). The p35GerDFR is DNA for expressing the Gerbera DFR gene in plant cells.

(2) Isolation of Gerbera ANS gene (GerANS)

[0094] RT-PCR was carried out by using the above cDNA as a template. Primers used for the PCR reaction were GerANS-F (5'-ATGGTGATTCAAGCAACCACA-3'; SEQ ID NO.: 78) and GerANS-R (5'-CTAGTTTTGCATCACT-TCGTCTTTAT-3'; SEQ ID NO.: 79) which were designed from a sequence (GenBank accession No.: AY997842) of a known Gerbera ANS gene (GerANS).

[0095] In the reaction, a step of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute and 10 seconds was repeated 45 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain Gerbera ANS gene (p35GerANS). The p35GerANS is DNA for expressing the Gerbera ANS gene in plant cells.

(3) Cotransfection with the Gerbera DFR gene and The GerANS gene

[0096] A petal of a white moth orchid (*Phal. amabilis*) was cotransfected with the respective genes of the CHS (PhCHS3), CHI (PhCHI1) and F3H (PhF3H1) which were derived from moth orchid, the F3'5'H gene (CcF3'5'H) which was derived from *Commelina communis* and the DFR gene (GerDFR) and ANS gene (GerANS) which were derived from Gerbera. As a result, deeper blue-purple newly emerged at the petal, as compared to Example 14.

EXAMPLE 16: Isolation of Torenia DFR gene and ANS gene and cotransfection therewith

(1) Isolation of Torenia DFR gene (TorDFR)

[0097] All RNA was isolated from a petal in a bud of a conventionally available Torenia (Torenia fournieri) by using RNeasy Plant Mini Kit (QIAGEN), and cDNA was prepared by using Superscriptll First-Strand Synthesis System (Invitrogen). RT-PCR was carried out by using this cDNA as a template. Primers used for the PCR reaction were an oligonucleotide TorDFR-F (5'-ATGAGCATGGAAGTAGTAGTACCA-3'; SEQ ID NO.: 80) and TorDFR-R (5'-CTATTCTATCT-TATGTTCTCCATGG-3'; SEQ ID NO.: 81) which were designed from a sequence (GenBank accession AB012924) of an already known Torenia DFR gene (TorDFR). In the reaction, a step of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute and 10 seconds was repeated 45 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain a Torenia DFR gene (p35TorDFR). The p35TorDFR is DNA for expressing the Torenia DFR gene in plant cells.

(2) Isolation of Torenia ANS gene (TorANS)

[0098] RT-PCR was carried out by using the above cDNA as a template. Primers used for the PCR reaction were TorANS-F (5'-ATGGTTTCTCCAGCATCTCCGA-3'; SEQ ID NO.: 82) and TorANS-R (5'-TCACTCAACACTCTTATCAT-CATGCTC-3'; SEQ ID NO.: 83) which were designed from a sequence (GenBank accession AB044091) of a known Torenia ANS gene (TorANS). In the reaction, a step of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute and 10 seconds was repeated 45 cycles. An obtained reaction product was subjected to cloning to SwaI site of pBS-P35T35 to obtain a Torenia ANS gene (p35TorANS). The p35TorANS is DNA for expressing ANS gene in plant cells.

(3) Cotransfection of Torenia DFR gene and Torenia ANS gene

[0099] A petal of a white moth orchid (*Phal. amabilis*) was cotransfected with respective genes of CHS (PhCHS3), CHI (PhCHI1) and F3H (PhF3H1) which were derived from moth orchid, the F3'5'H gene (CcF3'5'H) which was derived

from *Commelina communis* and the DFR gene (TorDFR) and ANS gene (TorANS) which were derived from Torenia in accordance with the method of Example 1, and as a result, cells having a deeper blue purple than that of Example 14 newly emerged at the petal.

EXAMINATION OF THE BEST DFR GENE AND ANS GENE

[0100] With respect to the changing white moth orchid to blue color, different genes derived from plants were introduced in order to find the best DFR gene and ANS gene, and such genes were compared and examined.

EXAMPLE 17: Observation of coloration of the petal cell

[0101] The coloration of the petal cells was observed by stereomicroscope SZX12 (OLYMPUS Corporation) and macroscopically observed. The standard of the degree of the coloration of the petal was that one which could be macroscopically observed was judged as "III", one which could be observed by a stereomicroscope with a magnification of at most 32 times was judged as "II", and one which could not be observed by a stereomicroscope with a magnification of at least 32 times was judged as "I", and one wherein coloration was not observed as judged as "-".

EXAMPLE 18: Comparison of the ANS genes in the petal of the white moth orchid

[0102] A petal of a white moth orchid (*Phal. amabilis*) was transfected with one of the following three sets of genes wherein only an ANS gene varied, and the degree of coloration was observed.

(1) Moth orchid ANS gene (PhANS1)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)*+Commelina communis* F3'5'H gene (CcF3'5'H)+Gerbera DFR gene (GerDFR)
(2) Gerbera ANS gene (GerANS)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+ *Commelina communis* F3'5'H gene (CcF3'5'H)+Gerbera DFR gene (GerDFR)
(3) Torenia ANS gene (TorANS)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+ *Commelina communis* F3'5'H gene (CcF3'5'H)+Gerbera DFR gene (GerDFR)

[0103] As a result, blue purple cells emerges on respective samples. The degrees of coloration of blue purple cells which emerged at twenty two pieces of the petal were compared. Results are shown on Fig. 3.

EXAMPLE 19: Comparison of the DFR genes in the petal of the white moth orchid

[0104] A petal of a white moth orchid (*Phal. amabilis*) was transfected with one of the following three sets of genes wherein only a DFR gene varied, and the degree of coloration was observed.

(1) Moth orchid DFR gene (Ph DFR)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+*Commelina communis* F3'5'H gene (CcF3'5'H)+ Torenea ANS gene (TorANS)
(2) Gerbera DFR gene (Ger DFR)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)*+Commelina communis* F3'5'H gene (CcF3'5'H)+Torenea ANS gene (TorANS)
(3) Torenia DFR gene (TorDFR)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+ *Commelina communis* F3'5'H gene (CcF3'5'H)+ Torenea ANS gene (TorANS)

[0105] As a result, blue purple cells emerges on respective samples. The degree of coloration of blue purple cells which emerged at twenty three pieces of the petal was compared. Results are shown on Fig. 4. When the PhDFR gene was used, the degree of blue-purple coloration was low, as compared to the case where the GerDFR gene or the TorDFR gene was used. It is considered that in order to produce deep blue-purple flower color, the Gerbera DFR gene (Ger DFR) and the Torenia DFR gene (TorDFR) are superior to the endogenous moth orchid DFR gene (PhDFR).

EXAMPLE 20: Performance of the *Commelina communis* F3'5'H gene

[0106] In order to measure the performance of the *Commelina communis* F3'5'H gene on the white moth orchid, comparative tests with the petunia gene was carried out.

[0107] A petal of a white moth orchid (*Phal. amabilis*) was cotransfected with the *Commelina communis* F3'5'H gene ((1) CcF3'5'H) or a petunia F3'5'H gene ((2) PetF3'5'H1, or (3) PetF3'5'H2), together with the CHS gene (PhCHS3), CHI gene (PhCHI1) and F3H gene (PhF3H1) which were derived from moth orchid and the DFR gene (GerDFR) and ANS gene (GerANS) which were derived from Gerbera.

[0108] As a result, in a case where the petunia F3'5'H gene (PetF3'5'H1) or the petunia F3'5'H2 gene (PetF3'5'H2) was used, blue coloration was little. On the other hand, in a case where the *Commelina communis* F3'5'H gene (CcF3'5'H) was used, clear coloration was observed (Fig. 5).

EXAMPLE 21: Necessity of gene transfection with F3H, CHI, CHS genes

[0109] Necessity of respective genes in the blue color change of white moth orchids was examined. A petal of a white moth orchid (*Phal. amabilis*) as a material was transfected with the following four types of gene sets, and the degree of coloration was observed.

(1) *Commelina communis* F3'5'H gene (CcF3'5'H)+Torenia DFR gene (TorDFR)+Torenia ANS gene (TorANS)
(2) Moth orchid F3H gene (PhF3H1)+*Commelina communis* F3'5'H gene (CcF3'5'H)+Torenia DFR gene (TorDFR) +Torenia ANS gene (TorANS)
(3) Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+*Commelina communis* F3'5'H gene (CcF3'5'H)+Torenia DFR gene (TorDFR)+Torenia ANS gene (TorANS)
(4) Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+*Commelina communis* F3'5'H gene (CcF3'5'H)+Torenia DFR gene (TorDFR)+Torenia ANS gene (TorANS)

[0110] A petal of *Phal. amabilis* was transfected with these genes, and the coloration of cells was observed. As a result, except (1), blue purple cells emerged. The degrees of coloration of 18 pieces of respective samples were compared, and results are shown in Fig. 6. From the results, it is evident that the F3H gene (PhF3H1) is essential for coloration.

EXAMPLE 22: Analysis of pigments emerged by transfecting a petal of white moth orchids

[0111] In the above experiment, whether pigments contained in the blue purple cells which emerged at the petal of the white moth-orchid (*Phal. amabilis*) was a delphinidin related pigment or not was examined. A petal of white moth orchid (*Phal. amabilis)* is cotransfected with the CHI gene, F3H gene and ANS gene which were derived from moth orchid, the DFR gene which was derived from Gerbera and the F3'5'H gene which was derived from *Commelina communis*, the petal was cultured for 5 days, and anthocyanidin was extracted from the petal in the same manner as in Example 6 and analyzed. As a result, delphinidin was detected as the main component of the pigment. The amount of the detected delphinidin was 16 ng per one sample (average of five samples). On the other hand, in the case of the group wherein the *Commelina communis* F3'5'H gene was excluded from the above gene sets, delphinidin was not detected. From the above result, it is evident that blue purple cells observed at the gene transfection of the white moth orchid were colored by a delphinidin derivative.

EXAMPLE 23: Application to white moth orchid other than *Phal. Amabilis*

[0112] A petal of a bud having a length of 1.7 cm of a pure white large moth orchid (*Phal. White Star*) was cotransfected with the CHS gene, CHI gene and F3H gene which were derived from moth orchid, the DFR gene and ANS gene which were derived from Gerbera and the F3'5'H gene which was derived from *Commelina communis* in accordance with the method of Example 1, and new blue purple cells emerged at the petal.

EXAMPLE 24: Preparation of DNA for moth orchid transformation by the Agrobacterium method

[0113] Transformation of moth orchids can be carried out by the Agrobacterium method, as well as the microprojectile bombardment method (Belarmino and Mii, Plant Cell Reports (2000) 19:435-442., Mishiba et al., Plant Cell Reports (2005) 24: 297-303). DNA for transformation to obtain gene recombinant plants by these methods was constructed. Maps and preparation procedures of respective plasmids are shown on Fig. 7 and 8.

(1) Construction of pBI-SAS1

[0114] A short fragment obtained by cutting pBS-SAS of Example 04 with NotI and HindIII was subjected to subcloning to a part that pBI-RHL described on PCT/JP02/12268 was cut with NotI and HindIII to construct pBI-SAS1. The pBI-SAS1 is a plasmid which imparts hygromycin resistance to plants by transformation with Agrobacterium.

(2) pBS-35S-FT construction

[0115] Since a period of at least one year is required for blossom of moth orchids, DNA for expressing flowering gene

FT (Kobayashi et al., Science (1999) 286: 1960-1962.) in moth orchids was constructed.

[0116] FTcDNA was prepared by amplifying all RNA prepared from whole plant of Arabidopsis by RT-PCR. For the PCR, AtFT 2nd-F (5'-GAAACCACCTGTTTGTTCAAGA-3';S EQ ID NO.: 84) and AtFT 2nd-R (5'-TCAATTGGT-TATAAAGGAAGAAGC-3'; SEQ: 85) were used as primer, a purified FT cDNA was inserted to a part formed by cutting pBS-P35T35 with SwaI, clone into which cDNA was inserted to a sense direction was selected to construct pBS-P35S-FT. The pBS-35S-FT is a plasmid of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator.

(3) Construction of pBIH-35S-CcF3'5'H

[0117] The p35CcF3'5'H was cut with BamHI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with AscI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted to a part formed by cutting pBI-SAS1 with AscI and SwaI to construct pBIH-35S-CcF3'5'H. The pBIH-35S-CcF3'5'H is a binary vector having a T-DNA region wherein HPT which is a selection marker and CcF3'5'H of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in this order.

(4) pBS-35S-mPhCHS3 construction

[0118] A nucleotide substitution without an amino acid substitution was introduced into a SphI cut part in the PhCHS3 cDNA to prepare a cDNA which could not be cut with SphI. PhCHS3-1038F (5'-GTAACATGTCGAGCGCTTGCGT-TCTTTTCATACTCG-3'; SEQ ID NO.: 86) and PhCHS3-1073R (5'-CGAGTATGAAAAGAACGCAAGCGCTCGACAT-GTTAC-3'; SEQ ID NO.: 87) were used as primers for the nucleotide substitution, and cDNA was synthesized by using the pBS-35S-PhCHS3 as a template and Pyrobest (Takara Bio Inc.). Then, the template plasmid was digested by DpnI treatment to construct pBS-35S-mPhCHS3.

(5) Construction of pBS-35S-UP1

[0119] After cutting p35PhCHI1 with AscI, and a cohesive end was blunted with Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting p35PhF3H with XbaI, blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-UP1. The pBS-35S-UP1 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of PhCHI1 and PhF3H1.

(6) Construction of pBS-35S-DelI

[0120] The p35TorANS was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with KpnI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting p35TorDFR with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with KpnI to construct pBS-35S-Del1. The pBS-35S-DelI is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of TorANS and TorDFR.

(7) Construction of pBS-35S-Del2

[0121] The p35CcF3'5'H was cut with AscI, a cohesive end was blunted with a Klenow fragment, and a DNA fragment was cutout by cutting the plasmid with SphI. This DNA fragment was inserted into a part formed by cutting the pBS-35S-DelI with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-Del2. The pBS-35S-Del2 is a plasmid in which respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator in the order of CcF3'5'H, TorANS and TorDFR.

(8) Construction of pBS-35S-Del8

[0122] The pBS-35S-UP1 was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-Del2 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-Del8. The pBS-35S-Del8 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of PhCHI1, PhF3H1, CcF3'5'H, TorANS and TorDFR.

(9) Construction of pBS-35S-Del9

[0123] The pBS-35S-mPhCHS3 was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-Del8 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-Del9. The pBS-35S-Del9 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of mPhCHS3, PhCHI1, PhF3H1, CcF3'5'H, TorANS and TorDFR.

(10) Construction of pBS-35S-Del15

[0124] The p35 CcF3'5'H was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-FT with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-Del15. The pBS-35S-Del15 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of CcF3'5'H and FT.

(11) Construction of pBS-35S-Del16

[0125] The p35 TorDFR was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting p35-PhANS1 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-355-Del16. The pBS-355-Del16 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of TorDFR and PhANS1.

(12) Construction of pBS-35S-UP4

[0126] The pBS-35S-Del16 was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-UP1 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-UP4. The pBS-35S-UP4 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of TorDFR, PhANS1, PhCHI1 and PhF3H1.

(13) Construction of pBS-35S-Del17

[0127] The p35 CcF3'5'H was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-UP4 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-35S-Del17. The pBS-35S-Del17 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of CcF3'5'H, TorDFR, PhANS1, PhCHI1 and PhF3H1.

(14) Construction of pBS-35S-Del18

[0128] The pBS-35S-Del15 was cut with AscI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with SphI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting the pBS-35S-UP4 with XbaI, and blunting a cohesive end with a Klenow fragment, followed by cutting with SphI to construct pBS-355-Del18. The pBS-35S-Del18 is a plasmid wherein respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of CcF3'5'H, FT, TorDFR, PhANS1, PhCHI1 and PhF3H1.

(15) Construction of pBIH-35S-Del8

[0129] The pBS-35S-Del8 was cut with SphI, a cohesive end was blunted with a Klenow fragment, followed by cutting with AscI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting pBI-SAS1 with AscI and SwaI, to construct pBIH-35S-Del8. The pBIH-35S-Del8 is a binary vector having a T-DNA region wherein HPT which is a selection marker and respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of PhCHI1, PhF3H1, CcF3'5'H, TorANS and TorDFR.

(16) Construction of pBIH-35S-Del9

[0130] The pBS-35S-Del9 was cut with SphI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with with AscI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting pBI-SAS1 with AscI and SwaI, to construct pBIH-35S-Del9. The pBIH-35S-Del9 is a binary vector having a T-DNA region wherein HPT which is a selection marker and respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of mPhCHS3, PhCHI1, PhF3H1, CcF3'5'H, TorANS and TorDFR.

(17) Construction of pBIH-355-Del17

[0131] The pBS-35S-Del17 was cut with SphI, and a cohesive end was blunted with a Klenow fragment, followed by cutting with AscI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting pBI-SAS1 with AscI and SwaI, to construct pBIH-35S-Del17. The pBIH-35S-Del17 is a binary vector having a T-DNA region wherein HPT which is a selection marker and respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of CcF3'5'H, TorDFR, PhANS1, PhCHI1 and PhF3H1.

(18) Construction of pBIH-35S-Del18

[0132] The pBS-35S-Del18 was cut with SphI, a cohesive end was blunted with a Klenow fragment, followed by cutting with AscI to cut out a DNA fragment from the plasmid. This DNA fragment was inserted into a part formed by cutting pBI-SAS1 with AscI and SwaI to construct pBIH-35S-Del18. The pBIH-35S-Del18 is a binary vector having a T-DNA region wherein HPT which is a selection marker and respective cDNA of which transcription is controlled by CaMV 35S promoter and CaMV 35S terminator are connected in the order of CcF3'5'H, FT, TorDFR, PhANS1, PhCHI1 and PhF3H1.

EXAMPLE 25: Production of transformed moth orchid

[0133] A transformed moth orchid produced by using DNA (pBIH-35S-CcF3'5'H, pBIH-35S-Del8, pBIH-35S-Del9, pBIH-35S-Del17 and pBIH-35S-Del18) which was constructed as binary vector in Example 24 and Agrobacterium EHA101 strain is selected with 50 mg/ml of hygromycin. As a result, a moth orchid wherein the genes shown in Example 24 are integrated into the chromosome could be obtained.

[0134] PLB can be induced from the obtained transformed moth orchid by using a part of plant such as flower stalk or axillary bud, and clone reproduction can be carried out. By carrying out cross-breeding using the obtained transformed moth orchid as a cross-breeding parent, it is possible to obtain a progeny having integrated genes.

[0135] Thus, by using the *Commelina communis* F3'5'H gene, a blue toned variety can be produced from an originally colored moth orchid, and a blue variety can be newly produced from a white moth orchid.

EXAMPLE 26: Evaluation of performance of the *Commelina communis* F3'5'H gene on a petal of Cymbidium

[0136] Ten pieces of a petal obtained from a bud having a length of 30 mm of a white Cymbidium (*Cym. Lovely angel 'The Two Virgins'*) were cotransfected with one of the following three sets of genes in accordance with the method of Example 1.

(1) *Commelina communis* F3'5'H gene (CcF3'5'H)+ Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+ Moth orchid F3H gene (PhF3H1)+Gerbera DFR gene (GerDFR)+Gerbera ANS gene (GerANS)
(2) Petunia F3'5'H2 gene (PetF3'5'H2)+Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+Gerbera DFR gene (GerDFR)+Gerbera ANS gene (GerANS)
(3) Moth orchid CHS gene (PhCHS3)+Moth orchid CHI gene (PhCHI1)+Moth orchid F3H gene (PhF3H1)+ Gerbera DFR gene (GerDFR)+Gerbera ANS gene (GerANS)

[0137] A transfected petal was static cultured for 5 days, and then observed by a stereomicroscope. Deep blue-purple cells newly emerged in the petals which was transfected with the gene set (1) including the *Commelina communis* F3'5'H gene (CcF3'5'H). On the other hand, in the petals which was transfected with the gene set (2) including the Petunia F3'5'H2 gene (PetF3'5'H2), light purple cells were observed only a part of the petals, and in the petals which was transfected with the gene set (3) containing no F3'5'H gene, flower color change was not observed at all. Results of evaluation of the degree of coloration of cells which emerged on each petal by the method described in Example 17 are shown on Fig. 9.

INDUSTRIAL APPLICABILITY

[0138] By using the *Commelina communis* F3'5'H gene, in addition to orchids having blue flowers which can not be accomplished by the conventional cross breeding methods, plants having various blue flower colors can be produced, and its applicability is extremely broad.

[0139] The entire disclosure of Japanese Patent Application No. 2007-066539 filed on March 15, 2007 including specification, claims, drawings and summary is incorporated herein by reference in its entirety.

SEQUENCE LISTING

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SEQ ID NO: 6, Primer 35FH-3
SEQ ID NO: 7, Primer C35FH-3
SEQ ID NO: 8, Primer C35FH-4
SEQ ID NO: 9, Primer C35FH-6
SEQ ID NO: 10, Primer C35FH-5
SEQ ID NO: 11, Primer C35FH-7
SEQ ID NO: 12, Primer C35FH-10
SEQ ID NO: 13, Primer C35FH-9
SEQ ID NO: 14, Oligonucleotide SAS-S
SEQ ID NO: 15, Oligonucleotide SAS-AS
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SEQ ID NO: 17, Primer T-CaMV35S-AscI-R
SEQ ID NO: 18, Primer CcF35H-F
SEQ ID NO: 19, Primer CcF35H-R
SEQ ID NO: 20, Primer PetF3'5'H1-F
SEQ ID NO: 21, Primer PetF3'5'H1-R
SEQ ID NO: 22, Primer PetF3'5'H2-F
SEQ ID NO: 23, Primer PetF3'5'H2-R
SEQ ID NO: 24, Primer PhCHS3 F1
SEQ ID NO: 25, Primer PhCHS3 R1
SEQ ID NO: 26, Nucleotide sequence encoding Doritaenopsis hybrid cultivar chalcone synthase
SEQ ID NO: 27, Amino acid sequence of Doritaenopsis hybrid cultivar chalcone synthase
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SEQ ID NO: 29, Primer CHI-dgR1
SEQ ID NO: 30, Primer CHI-dgF3
SEQ ID NO: 31, Primer CHI-dgR3
SEQ ID NO: 32, Primer PhCHI-GSP F1
SEQ ID NO: 33, Primer PhCHI-GSP F2
SEQ ID NO: 34, Primer PhCHI-GSP R1
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SEQ ID NO: 36, Primer PhCHI init
SEQ ID NO: 37, Primer PhCHI term
SEQ ID NO: 38, Nucleotide sequence encoding Doritaenopsis hybrid cultivar chalcone isomerase
SEQ ID NO: 39, Amino acid sequence of Doritaenopsis hybrid cultivar chalcone isomerase
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SEQ ID NO: 41, Primer F3H-dgR1
SEQ ID NO: 42, Primer F3H-dgF3
SEQ ID NO: 43, Primer F3H-dgR3
SEQ ID NO: 44, Primer PhF3H-GSPF1
SEQ ID NO: 45, Primer PhF3H-GSPF2
SEQ ID NO: 46, Primer PhF3H-GSPR1

SEQ ID NO: 47, Primer PhF3H-GSPR2
SEQ ID NO: 48, Primer PhF3H init.
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SEQ ID NO: 55, Primer DFRD-R2
SEQ ID NO: 56, Primer DFRD-F3
SEQ ID NO: 57, Primer DFRD-R3
SEQ ID NO: 58, Primer PhDFR-F1
SEQ ID NO: 59, Primer PhDFR-F2
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SEQ ID NO: 62, Primer PhDFR-F8A5
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SEQ ID NO: 65, Amino acid sequence of Doritaenopsis hybrid cultivar dihydroflavonol 4-reductase
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SEQ ID NO: 71, Primer PhANS5RACEGSP2
SEQ ID NO: 72, Primer PhANS init
SEQ ID NO: 73, Primer PhANS term
SEQ ID NO: 74, Nucleotide sequence encoding Doritaenopsis hybrid cultivar anthocyanidin synthase
SEQ ID NO: 75, Amino acid sequence of Doritaenopsis hybrid cultivar anthocyanidin synthase
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SEQ ID NO: 77, Primer GerDFR-R
SEQ ID NO: 78, Primer GerANS-F
SEQ ID NO: 79, Primer GerANS-R
SEQ ID NO: 80, Primer TorDFR-F
SEQ ID NO: 81, Primer TorDFR-R
SEQ ID NO: 82, Primer TorANS-F
SEQ ID NO: 83, Primer TorANS-R
SEQ ID NO: 84, Primer AtFT 2nd-F
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Pro Ala Ala Val Leu Ala Ile Gly Thr Ser Thr Pro Pro Asn Ala Leu
20 25 30

tat cag gcc gat tat ccc gat tat tac ttc aga atc acc aac tgc gag 144
Tyr Gln Ala Asp Tyr Pro Asp Tyr Tyr Phe Arg Ile Thr Asn Cys Glu
35 40 45

cat ctc act gac ctc aag gag aag ttc aag cga atg tgc gag aaa tcc 192
His Leu Thr Asp Leu Lys Glu Lys Phe Lys Arg Met Cys Glu Lys Ser
50 55 60

atg ata aaa aaa cgg tac atg tat cta aca gaa gaa ttc ctg aaa gaa 240
Met Ile Lys Lys Arg Tyr Met Tyr Leu Thr Glu Glu Phe Leu Lys Glu
65 70 75 80

aat ccc aat atc tgc gca ttc atg gct cct tca ctc gac gcc cgg caa 288
Asn Pro Asn Ile Cys Ala Phe Met Ala Pro Ser Leu Asp Ala Arg Gln
85 90 95

```
gac ata gtt gtc gcc gag gtc ccg aag ctc gcc aaa gag gcc gcc gcg      336
Asp Ile Val Val Ala Glu Val Pro Lys Leu Ala Lys Glu Ala Ala Ala
            100                 105                 110

cgc gcc atc aag gaa tgg gga cac ccc aaa tca cgc ata act cat ctc      384
Arg Ala Ile Lys Glu Trp Gly His Pro Lys Ser Arg Ile Thr His Leu
        115                 120                 125

atc ttc tgc acc acc agc ggc gtc gac atg ccc ggc gcc gac tac caa      432
Ile Phe Cys Thr Thr Ser Gly Val Asp Met Pro Gly Ala Asp Tyr Gln
    130                 135                 140

ctc acc cgc ctc ctc ggt ctc cgc ccc tcc gtc aac aga ttc atg ctc      480
Leu Thr Arg Leu Leu Gly Leu Arg Pro Ser Val Asn Arg Phe Met Leu
145                 150                 155                 160

tac cag cag ggc tgc ttc gcc ggc ggc acc gtc ctc cgc ctc gcc aag      528
Tyr Gln Gln Gly Cys Phe Ala Gly Gly Thr Val Leu Arg Leu Ala Lys
                165                 170                 175

gat ctc gcc gag aac aac gcc ggc gcc cgc gtg ctc gtc gtt tgc tcc      576
Asp Leu Ala Glu Asn Asn Ala Gly Ala Arg Val Leu Val Val Cys Ser
            180                 185                 190

gaa atc acc gcc gtc act ttc cgc ggc ccg tcg gaa tcc cat ctc gat      624
Glu Ile Thr Ala Val Thr Phe Arg Gly Pro Ser Glu Ser His Leu Asp
        195                 200                 205

tcc ctc gtc gga cag gcg ctc ttc ggc gac ggc gcc gcc gct atc att      672
Ser Leu Val Gly Gln Ala Leu Phe Gly Asp Gly Ala Ala Ala Ile Ile
    210                 215                 220

gtc gga tcc gac cct gat tta gcc acc gag cgc cct ctg ttt caa cta      720
Val Gly Ser Asp Pro Asp Leu Ala Thr Glu Arg Pro Leu Phe Gln Leu
225                 230                 235                 240

gtc tct gct tcc caa acc atc ctt ccc gaa tca gag ggc gcc att gat      768
Val Ser Ala Ser Gln Thr Ile Leu Pro Glu Ser Glu Gly Ala Ile Asp
                245                 250                 255

ggc cac ctt cgt gaa atc ggg ctc acc ttc cac cta ctc aaa gac gtc      816
Gly His Leu Arg Glu Ile Gly Leu Thr Phe His Leu Leu Lys Asp Val
            260                 265                 270

ccc ggc ctc att tct aaa aac att caa aaa tgt ctc ctt gag gcc ttc      864
Pro Gly Leu Ile Ser Lys Asn Ile Gln Lys Cys Leu Leu Glu Ala Phe
        275                 280                 285
```

```
aag cca ctt ggt gtg ctt gat tgg aac tct att ttt tgg atc gct cac      912
Lys Pro Leu Gly Val Leu Asp Trp Asn Ser Ile Phe Trp Ile Ala His
    290                 295                 300

ccg ggc ggc ccg gct ata ctc gat caa gtt gag acc aag ctc ggt cta      960
Pro Gly Gly Pro Ala Ile Leu Asp Gln Val Glu Thr Lys Leu Gly Leu
305                 310                 315                 320

aag tcc gag aag ctc gcc gcg agt aga aat gtg ctc gct gac tac ggt     1008
Lys Ser Glu Lys Leu Ala Ala Ser Arg Asn Val Leu Ala Asp Tyr Gly
                325                 330                 335

aac atg tcg agc gca tgc gtt ctt ttc ata ctc gat gag atg cga agg     1056
Asn Met Ser Ser Ala Cys Val Leu Phe Ile Leu Asp Glu Met Arg Arg
            340                 345                 350

cga tcg gca gag gct ggg cag tcg acc act ggc gag ggt ttg gag tgg     1104
Arg Ser Ala Glu Ala Gly Gln Ser Thr Thr Gly Glu Gly Leu Glu Trp
        355                 360                 365

gga gtt cta ttc ggg ttc ggt ccg gga ctt acg gtc gag act gtt gta     1152
Gly Val Leu Phe Gly Phe Gly Pro Gly Leu Thr Val Glu Thr Val Val
    370                 375                 380

tta cgc agc gtt ccg att ggt ggc acc gag taa                        1185
Leu Arg Ser Val Pro Ile Gly Gly Thr Glu
385                 390
```

<210> 27
<211> 394
<212> PRT
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Sogo Vivien x Doritaenopsis Sogo Yenlin)

<400> 27

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Met Ala Pro Ala Met Glu Glu Ile Arg Arg Thr Gln Arg Ala Glu Gly
1               5                   10                  15

Pro Ala Ala Val Leu Ala Ile Gly Thr Ser Thr Pro Pro Asn Ala Leu
            20                  25                  30

Tyr Gln Ala Asp Tyr Pro Asp Tyr Tyr Phe Arg Ile Thr Asn Cys Glu
        35                  40                  45
```

His Leu Thr Asp Leu Lys Glu Lys Phe Lys Arg Met Cys Glu Lys Ser
50 55 60

Met Ile Lys Lys Arg Tyr Met Tyr Leu Thr Glu Glu Phe Leu Lys Glu
65 70 75 80

Asn Pro Asn Ile Cys Ala Phe Met Ala Pro Ser Leu Asp Ala Arg Gln
85 90 95

Asp Ile Val Val Ala Glu Val Pro Lys Leu Ala Lys Glu Ala Ala Ala
100 105 110

Arg Ala Ile Lys Glu Trp Gly His Pro Lys Ser Arg Ile Thr His Leu
115 120 125

Ile Phe Cys Thr Thr Ser Gly Val Asp Met Pro Gly Ala Asp Tyr Gln
130 135 140

Leu Thr Arg Leu Leu Gly Leu Arg Pro Ser Val Asn Arg Phe Met Leu
145 150 155 160

Tyr Gln Gln Gly Cys Phe Ala Gly Gly Thr Val Leu Arg Leu Ala Lys
165 170 175

Asp Leu Ala Glu Asn Asn Ala Gly Ala Arg Val Leu Val Val Cys Ser
180 185 190

Glu Ile Thr Ala Val Thr Phe Arg Gly Pro Ser Glu Ser His Leu Asp
195 200 205

Ser Leu Val Gly Gln Ala Leu Phe Gly Asp Gly Ala Ala Ala Ile Ile
210 215 220

Val Gly Ser Asp Pro Asp Leu Ala Thr Glu Arg Pro Leu Phe Gln Leu
225 230 235 240

Val Ser Ala Ser Gln Thr Ile Leu Pro Glu Ser Glu Gly Ala Ile Asp
245 250 255

Gly His Leu Arg Glu Ile Gly Leu Thr Phe His Leu Leu Lys Asp Val
260 265 270

Pro Gly Leu Ile Ser Lys Asn Ile Gln Lys Cys Leu Leu Glu Ala Phe
275 280 285

Lys Pro Leu Gly Val Leu Asp Trp Asn Ser Ile Phe Trp Ile Ala His
290 295 300

Pro Gly Gly Pro Ala Ile Leu Asp Gln Val Glu Thr Lys Leu Gly Leu
305 310 315 320

Lys Ser Glu Lys Leu Ala Ala Ser Arg Asn Val Leu Ala Asp Tyr Gly
325 330 335

Asn Met Ser Ser Ala Cys Val Leu Phe Ile Leu Asp Glu Met Arg Arg
340 345 350

Arg Ser Ala Glu Ala Gly Gln Ser Thr Thr Gly Glu Gly Leu Glu Trp
355 360 365

Gly Val Leu Phe Gly Phe Gly Pro Gly Leu Thr Val Glu Thr Val Val
370 375 380

Leu Arg Ser Val Pro Ile Gly Gly Thr Glu
385 390

<210> 28
<211> 26
<212> DNA
<213> Artificial

<220>

<223> Primer CHI-dgF1

<400> 28
ttyctcgsyg gbgcmggygw vmgvgg 26

<210> 29
<211> 28
<212> DNA
<213> Artificial

<220>
<223> Primer CHI-dgR1

<220>
<221> modified_base
<222> (5)..(20)
<223> n(5, 8, 20) = i

<400> 29
cmggnganac vscrtkytyn ccratvat 28

<210> 30
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer CHI-dgF3

<220>
<221> modified_base
<222> (3)..(14)
<223> n(3, 11, 14) = i

<400> 30
tmnkywcmgg nsmnttygar aaryt 25

<210> 31
<211> 26
<212> DNA
<213> Artificial

<220>
<223> Primer CHI-dgR3

<220>
<221> modified_base
<222> (3)..(21)
<223> n (3, 12, 21)=i

<400> 31
tynccratva tngwhtccar naybgc 26

<210> 32
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Primer PhCHI-GSP F1

<400> 32
atgctgctgc cattaacggg tca 23

<210> 33
<211> 22
<212> DNA
<213> Artificial

<220>
<223> Primer PhCHI-GSP F2

<400> 33
tccgagaagg tctccgggaa ct 22

<210> 34
<211> 24
<212> DNA
<213> Artificial

<220>
<223> Primer PhCHI-GSP R1

<400> 34
gcattcgtca gcttcttgct ctct 24

<210> 35
<211> 21
<212> DNA

<213> Artificial

<220>
<223> Primer PhCHI-GSP R2

<400> 35
atcacatcag tctcagccac a 21

<210> 36
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer PhCHI init

<400> 36
atggcagaaa cagtggcgac gccca 25

<210> 37
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer PhCHI term

<400> 37
tcaaacgact ccatcttgct c 21

<210> 38
<211> 723
<212> DNA
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Sogo Vivien x Doritaenopsis Sogo Yenlin)

<220>
<221> CDS
<222> (1).. (723)
<223> Doritaenopsis hybrid cultivar chalcone isomerase (PhCHI 3)

<400> 38
atg gca gaa aca gtg gcg acg ccc atc gag gtg gag gga gtg aag ttt 48
Met Ala Glu Thr Val Ala Thr Pro Ile Glu Val Glu Gly Val Lys Phe
1 5 10 15

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ccg gcc gag atc tcg tcg ccg gcg acc tcg aaa cct cta ttt ctc ggt      96
Pro Ala Glu Ile Ser Ser Pro Ala Thr Ser Lys Pro Leu Phe Leu Gly
            20                  25                  30

ggc gca ggg gcg agg ggt ata gaa gtt gga gga aag ttt tta gcc gta     144
Gly Ala Gly Ala Arg Gly Ile Glu Val Gly Gly Lys Phe Leu Ala Val
        35                  40                  45

acc gcg atc gga gtg tac ttg gaa gcg gcg gtg att ccg gcg atc gcc     192
Thr Ala Ile Gly Val Tyr Leu Glu Ala Ala Val Ile Pro Ala Ile Ala
    50                  55                  60

gga aaa tgg acg ggg aag aag gcg gag aag ctc act gat tcg gtt gac     240
Gly Lys Trp Thr Gly Lys Lys Ala Glu Lys Leu Thr Asp Ser Val Asp
65                  70                  75                  80

ttt tac cga gac att att aca ggt tcc ttt gag aag ctg acg aga gtg     288
Phe Tyr Arg Asp Ile Ile Thr Gly Ser Phe Glu Lys Leu Thr Arg Val
                85                  90                  95

acg atg ctg ctg cca tta acg ggt caa cag tac tcc gag aag gtc tcc     336
Thr Met Leu Leu Pro Leu Thr Gly Gln Gln Tyr Ser Glu Lys Val Ser
            100                 105                 110

ggg aac tgc gtc gcc gca tgg aaa gcc gcc gga gaa tac aca gag gaa     384
Gly Asn Cys Val Ala Ala Trp Lys Ala Ala Gly Glu Tyr Thr Glu Glu
        115                 120                 125

gaa gca acg gcc att aat aag ttt ctg gaa atc ttc aag cct aag aac     432
Glu Ala Thr Ala Ile Asn Lys Phe Leu Glu Ile Phe Lys Pro Lys Asn
    130                 135                 140

ttt ctt cca ggc acc tcc atc atc ttc act cat tcc cct cat ggc tct     480
Phe Leu Pro Gly Thr Ser Ile Ile Phe Thr His Ser Pro His Gly Ser
145                 150                 155                 160

ctc act att gga ttt ttg gag ggg gat ggc gtt cct gtg gct gag act     528
Leu Thr Ile Gly Phe Leu Glu Gly Asp Gly Val Pro Val Ala Glu Thr
                165                 170                 175

gat gtg ata gag agc aag aag ctg acg aat gcg gtg ttg gaa tcc att     576
Asp Val Ile Glu Ser Lys Lys Leu Thr Asn Ala Val Leu Glu Ser Ile
            180                 185                 190

ata ggg gag aat gga gtt tct ccc gct gcg aaa cag agc ctg gct cgg     624
Ile Gly Glu Asn Gly Val Ser Pro Ala Ala Lys Gln Ser Leu Ala Arg
        195                 200                 205
```

agg ttt tca gag ctt ctg aat aag aaa gaa gac caa gaa gaa gaa gat 672
Arg Phe Ser Glu Leu Leu Asn Lys Lys Glu Asp Gln Glu Glu Glu Asp
210 215 220

ggg att ttg gat gtg gag aaa gcc aaa tta gag caa gat gga gtc gtt 720
Gly Ile Leu Asp Val Glu Lys Ala Lys Leu Glu Gln Asp Gly Val Val
225 230 235 240

tga 723

<210> 39
<211> 240
<212> PRT
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Sogo Vivien x Doritaenopsis Sogo Yenlin)

<400> 39

Met Ala Glu Thr Val Ala Thr Pro Ile Glu Val Glu Gly Val Lys Phe
1 5 10 15

Pro Ala Glu Ile Ser Ser Pro Ala Thr Ser Lys Pro Leu Phe Leu Gly
20 25 30

Gly Ala Gly Ala Arg Gly Ile Glu Val Gly Gly Lys Phe Leu Ala Val
35 40 45

Thr Ala Ile Gly Val Tyr Leu Glu Ala Ala Val Ile Pro Ala Ile Ala
50 55 60

Gly Lys Trp Thr Gly Lys Lys Ala Glu Lys Leu Thr Asp Ser Val Asp
65 70 75 80

Phe Tyr Arg Asp Ile Ile Thr Gly Ser Phe Glu Lys Leu Thr Arg Val
85 90 95

Thr Met Leu Leu Pro Leu Thr Gly Gln Gln Tyr Ser Glu Lys Val Ser
100 105 110

Gly Asn Cys Val Ala Ala Trp Lys Ala Ala Gly Glu Tyr Thr Glu Glu
115 120 125

Glu Ala Thr Ala Ile Asn Lys Phe Leu Glu Ile Phe Lys Pro Lys Asn
130 135 140

Phe Leu Pro Gly Thr Ser Ile Ile Phe Thr His Ser Pro His Gly Ser
145 150 155 160

Leu Thr Ile Gly Phe Leu Glu Gly Asp Gly Val Pro Val Ala Glu Thr
165 170 175

Asp Val Ile Glu Ser Lys Lys Leu Thr Asn Ala Val Leu Glu Ser Ile
180 185 190

Ile Gly Glu Asn Gly Val Ser Pro Ala Ala Lys Gln Ser Leu Ala Arg
195 200 205

Arg Phe Ser Glu Leu Leu Asn Lys Lys Glu Asp Gln Glu Glu Glu Asp
210 215 220

Gly Ile Leu Asp Val Glu Lys Ala Lys Leu Glu Gln Asp Gly Val Val
225 230 235 240

<210> 40
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer F3H-dgF1

<220>
<221> modified_base
<222> (2)..(23)
<223> n(2, 5, 23) = i

<400> 40
tnvgngayga rgabgarmgb ccnaa 25

<210> 41
<211> 26
<212> DNA
<213> Artificial

<220>
<223> Primer F3H-dgR1

<400> 41
acbgcyygrt grtchgcrtt cttraa 26

<210> 42
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer F3H-dgF3

<220>
<221> modified_base
<222> (24)..(24)
<223> n(24) = i

<400> 42
aarytbrgkt tygayatgwc hggng 25

<210> 43
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Primer F3H-dgR3

<220>
<221> modified_base
<222> (12)..(12)
<223> n(12) = d

<220>
<221> modified_base
<222> (18)..(18)
<223> n(18) = i

<400> 43
gghwsracvg tnatccangw btt 23

<210> 44
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H-GSPF1

<400> 44
ttctcatacc caatcgggag 20

<210> 45
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H-GSPF2

<400> 45
aatcgggagc cgcgattact 20

<210> 46
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H-GSPR1

<400> 46
tctgtgtggc gcttcaggcc 20

<210> 47
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H-GSPR2

<400> 47
tgaggtccgg ttgcgggcat ttt 23

<210> 48
<211> 25
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H init.

<400> 48
atggccccaa taccattcct accga 25

<210> 49
<211> 35
<212> DNA
<213> Artificial

<220>
<223> Primer PhF3H term.

<400> 49
ccttaagcta aaatctcatt taatgccttt gctcc 35

<210> 50
<211> 1113
<212> DNA
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Sogo Vivien x Doritaenopsis Sogo Yenlin)

<220>
<221> CDS
<222> (1).. (1113)
<223> Doritaenopsis hybrid cultivar flavanone 3-hydroxylase (PhF3H 1)

<400> 50
atg gcc cca ata cca ttc cta ccg act gcg gtt aca gag aag aca ctg 48
Met Ala Pro Ile Pro Phe Leu Pro Thr Ala Val Thr Glu Lys Thr Leu
1 5 10 15

aga gca agc ttt gta cgg gat gag gac gag agg cca aag gta gcc tac 96
Arg Ala Ser Phe Val Arg Asp Glu Asp Glu Arg Pro Lys Val Ala Tyr
20 25 30

```
aac gaa ttc agt aac cag att ccg gtg atc tca ctt cag ggg atc gaa      144
Asn Glu Phe Ser Asn Gln Ile Pro Val Ile Ser Leu Gln Gly Ile Glu
        35                  40                  45

gag aat gga gac gga ggt cga agg tcg gag att tgc cgg agt atc gtg      192
Glu Asn Gly Asp Gly Gly Arg Arg Ser Glu Ile Cys Arg Ser Ile Val
    50                  55                  60

gca gcg tgc gag gac tgg gga atc ttt cag gcc gtc gac cat ggt gtc      240
Ala Ala Cys Glu Asp Trp Gly Ile Phe Gln Ala Val Asp His Gly Val
65                  70                  75                  80

gat gca ggg ctc atc gca gac atg aac cgc ctt gct cga gag ttc ttc      288
Asp Ala Gly Leu Ile Ala Asp Met Asn Arg Leu Ala Arg Glu Phe Phe
                85                  90                  95

gat ctg ctg cca gag gag aag ctt cgt ttt gac atg tcc ggc ggg aag      336
Asp Leu Leu Pro Glu Glu Lys Leu Arg Phe Asp Met Ser Gly Gly Lys
            100                 105                 110

aaa ggc ggc ttc atc gtt tcc agc cat ctt cag ggt gaa gta gtt caa      384
Lys Gly Gly Phe Ile Val Ser Ser His Leu Gln Gly Glu Val Val Gln
        115                 120                 125

gat tgg agg gag atc gtt acc tat ttc tca tac cca atc ggg agc cgc      432
Asp Trp Arg Glu Ile Val Thr Tyr Phe Ser Tyr Pro Ile Gly Ser Arg
    130                 135                 140

gat tac tcg cgg tgg ccg gac aag ccg gag ggg tgg cgc gct gtt gtg      480
Asp Tyr Ser Arg Trp Pro Asp Lys Pro Glu Gly Trp Arg Ala Val Val
145                 150                 155                 160

gag gag tac agc gcc aag ctg atg gag ctg gcc tgc aat ctc ctc ggc      528
Glu Glu Tyr Ser Ala Lys Leu Met Glu Leu Ala Cys Asn Leu Leu Gly
                165                 170                 175

gtg cta tcg gaa gcc atg gga cta gat cgt gaa gcc cta gcc gga gcc      576
Val Leu Ser Glu Ala Met Gly Leu Asp Arg Glu Ala Leu Ala Gly Ala
            180                 185                 190

tgt atc gat atg gac cag aaa ttg gtg gtc aat ttc tac cca aaa tgc      624
Cys Ile Asp Met Asp Gln Lys Leu Val Val Asn Phe Tyr Pro Lys Cys
        195                 200                 205

ccg caa ccg gac ctc acc ctg ggc ctg aag cgc cac aca gac ccc ggc      672
Pro Gln Pro Asp Leu Thr Leu Gly Leu Lys Arg His Thr Asp Pro Gly
    210                 215                 220
```

```
acc att acc ctg ttg ctt caa gat caa gtc ggc ggt ctc caa gcc acc      720
Thr Ile Thr Leu Leu Leu Gln Asp Gln Val Gly Gly Leu Gln Ala Thr
225                 230                 235                 240

aag gac gac ggt aaa acc tgg atc acc gtt cag cct gtc cag aat gct      768
Lys Asp Asp Gly Lys Thr Trp Ile Thr Val Gln Pro Val Gln Asn Ala
                245                 250                 255

ttc gtc gtt aac ctc ggc gac cac ggt cat tac ctg agc aac ggt cgg      816
Phe Val Val Asn Leu Gly Asp His Gly His Tyr Leu Ser Asn Gly Arg
            260                 265                 270

ttt aag aac gcg gac cat cag gcc gtc gtg aac tcg aat tac agc cgg      864
Phe Lys Asn Ala Asp His Gln Ala Val Val Asn Ser Asn Tyr Ser Arg
        275                 280                 285

ctt tcg atc gcg gcg ttc cag aac cct gct ccg gaa gcg gtt gtt tac      912
Leu Ser Ile Ala Ala Phe Gln Asn Pro Ala Pro Glu Ala Val Val Tyr
    290                 295                 300

ccg cta gcg gtg agg gaa gga gag agg ccg gtg atg gag gag ggc atc      960
Pro Leu Ala Val Arg Glu Gly Glu Arg Pro Val Met Glu Glu Gly Ile
305                 310                 315                 320

aca ttt gcg gag atg tat agg agg aag atg agc agg gat ctg gag ctg     1008
Thr Phe Ala Glu Met Tyr Arg Arg Lys Met Ser Arg Asp Leu Glu Leu
                325                 330                 335

gcc agg ttg aag aag atg gcg aag atg gag agt ggg gag gaa ggg gcc     1056
Ala Arg Leu Lys Lys Met Ala Lys Met Glu Ser Gly Glu Glu Gly Ala
            340                 345                 350

gca gga aag act gct gag gtt act gga gca aag gca tta aat gag att     1104
Ala Gly Lys Thr Ala Glu Val Thr Gly Ala Lys Ala Leu Asn Glu Ile
        355                 360                 365

tta gct taa                                                        1113
Leu Ala
    370
```

<210> 51
<211> 370
<212> PRT
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Sogo Vivien x Doritaenopsis Sogo Yenlin)

<400> 51

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Met Ala Pro Ile Pro Phe Leu Pro Thr Ala Val Thr Glu Lys Thr Leu
1               5                  10                  15

Arg Ala Ser Phe Val Arg Asp Glu Asp Glu Arg Pro Lys Val Ala Tyr
            20                  25                  30

Asn Glu Phe Ser Asn Gln Ile Pro Val Ile Ser Leu Gln Gly Ile Glu
        35                  40                  45

Glu Asn Gly Asp Gly Gly Arg Arg Ser Glu Ile Cys Arg Ser Ile Val
    50                  55                  60

Ala Ala Cys Glu Asp Trp Gly Ile Phe Gln Ala Val Asp His Gly Val
65                  70                  75                  80

Asp Ala Gly Leu Ile Ala Asp Met Asn Arg Leu Ala Arg Glu Phe Phe
                85                  90                  95

Asp Leu Leu Pro Glu Glu Lys Leu Arg Phe Asp Met Ser Gly Gly Lys
            100                 105                 110

Lys Gly Gly Phe Ile Val Ser Ser His Leu Gln Gly Glu Val Val Gln
        115                 120                 125

Asp Trp Arg Glu Ile Val Thr Tyr Phe Ser Tyr Pro Ile Gly Ser Arg
    130                 135                 140

Asp Tyr Ser Arg Trp Pro Asp Lys Pro Glu Gly Trp Arg Ala Val Val
145                 150                 155                 160

Glu Glu Tyr Ser Ala Lys Leu Met Glu Leu Ala Cys Asn Leu Leu Gly
                165                 170                 175

Val Leu Ser Glu Ala Met Gly Leu Asp Arg Glu Ala Leu Ala Gly Ala
            180                 185                 190
```

Cys Ile Asp Met Asp Gln Lys Leu Val Val Asn Phe Tyr Pro Lys Cys
195 200 205

Pro Gln Pro Asp Leu Thr Leu Gly Leu Lys Arg His Thr Asp Pro Gly
210 215 220

Thr Ile Thr Leu Leu Leu Gln Asp Gln Val Gly Gly Leu Gln Ala Thr
225 230 235 240

Lys Asp Asp Gly Lys Thr Trp Ile Thr Val Gln Pro Val Gln Asn Ala
245 250 255

Phe Val Val Asn Leu Gly Asp His Gly His Tyr Leu Ser Asn Gly Arg
260 265 270

Phe Lys Asn Ala Asp His Gln Ala Val Val Asn Ser Asn Tyr Ser Arg
275 280 285

Leu Ser Ile Ala Ala Phe Gln Asn Pro Ala Pro Glu Ala Val Val Tyr
290 295 300

Pro Leu Ala Val Arg Glu Gly Glu Arg Pro Val Met Glu Glu Gly Ile
305 310 315 320

Thr Phe Ala Glu Met Tyr Arg Arg Lys Met Ser Arg Asp Leu Glu Leu
325 330 335

Ala Arg Leu Lys Lys Met Ala Lys Met Glu Ser Gly Glu Glu Gly Ala
340 345 350

Ala Gly Lys Thr Ala Glu Val Thr Gly Ala Lys Ala Leu Asn Glu Ile
355 360 365

Leu Ala
370

<210> 52
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-F1

<220>
<221> modified_base
<222> (9).. (12)
<223> n (9, 12) =i

<400> 52
ttycaygtng cnacnccnat g 21

<210> 53
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-R1

<220>
<221> modified_base
<222> (1).. (1)
<223> n (1) = d

<400> 53
natngcrtcr tcraacatyt c 21

<210> 54
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-F2

<220>
<221> modified_base
<222> (15).. (15)
<223> n (15) = i

<400> 54
atgaayttyc arwsnrarga ycc 23

<210> 55
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-R2

<220>
<221> modified_base
<222> (4)..(15)
<223> n(4, 10, 15) = i

<400> 55
rcanatrtan ckncnrttng c 21

<210> 56
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-F3

<400> 56
garaaygarg tnathaarcc 20

<210> 57
<211> 21
<212> DNA
<213> Artificial

<220>
<223> Primer DFRD-R3

<220>
<221> modified_base
<222> (7)..(7)
<223> n(7) = i

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<210> 64
<211> 1140
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<220>
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<222> (1).. (1140)
<223> Doritaenopsis hybrid cultivar dihydroflavonol 4-reductase (PhDFR)

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1 5 10 15

tac gtg ggt tca tgg ctg gtt atg aag ctt ctt cga aag ggt tat gag 96
Tyr Val Gly Ser Trp Leu Val Met Lys Leu Leu Arg Lys Gly Tyr Glu
20 25 30

gtc agg gct aca gtc aga gat cca aca aat tct aaa aaa gtg aag ccg 144
Val Arg Ala Thr Val Arg Asp Pro Thr Asn Ser Lys Lys Val Lys Pro

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          35                  40                  45

ttg ttg gat ctt ccg ggc tcg aat gaa ctg ctc agc ata tgg aaa gca      192
Leu Leu Asp Leu Pro Gly Ser Asn Glu Leu Leu Ser Ile Trp Lys Ala
    50                  55                  60

gat cta aat gac att gaa ggg agc ttc gat gag gtg ata cgt ggt tgt      240
Asp Leu Asn Asp Ile Glu Gly Ser Phe Asp Glu Val Ile Arg Gly Cys
65                  70                  75                  80

gtt ggg gtg ttc cat gtc gct act ccc atg aat ttt caa tcc aaa gac      288
Val Gly Val Phe His Val Ala Thr Pro Met Asn Phe Gln Ser Lys Asp
                85                  90                  95

cct gag aac gaa gtg ata caa ccg gca atc aac ggt ttg ctg agc atc      336
Pro Glu Asn Glu Val Ile Gln Pro Ala Ile Asn Gly Leu Leu Ser Ile
            100                 105                 110

ctg agg tca tgc aaa agg tcg ggc agc gta agg cgc gtg atc ttc aca      384
Leu Arg Ser Cys Lys Arg Ser Gly Ser Val Arg Arg Val Ile Phe Thr
        115                 120                 125

tct tcc gca gga aca gtc aac gtg gag gaa cgc cga gca ccg gtg tac      432
Ser Ser Ala Gly Thr Val Asn Val Glu Glu Arg Arg Ala Pro Val Tyr
    130                 135                 140

gac gag agc tcc tgg agc gac ctc gat ttc atc acc cgt gtc aaa atg      480
Asp Glu Ser Ser Trp Ser Asp Leu Asp Phe Ile Thr Arg Val Lys Met
145                 150                 155                 160

acc ggt tgg atg tac ttc gta tca aaa aca ctt gcg gag aag gct gct      528
Thr Gly Trp Met Tyr Phe Val Ser Lys Thr Leu Ala Glu Lys Ala Ala
                165                 170                 175

tgg gag ttt gtg aaa gaa aat gac gtt gat ttt ata gcc ata att ccc      576
Trp Glu Phe Val Lys Glu Asn Asp Val Asp Phe Ile Ala Ile Ile Pro
            180                 185                 190

act ttg gtg gtg ggt tcc ttc ata aca gat gag atg ccg cca agt ttg      624
Thr Leu Val Val Gly Ser Phe Ile Thr Asp Glu Met Pro Pro Ser Leu
        195                 200                 205

acc act gca ttt tca tta att aca gga aat gaa gct cat tac tcg ata      672
Thr Thr Ala Phe Ser Leu Ile Thr Gly Asn Glu Ala His Tyr Ser Ile
    210                 215                 220

ata aag caa gct caa ttt gtt cat ttg gat gac tta tgt gat gct cat      720
Ile Lys Gln Ala Gln Phe Val His Leu Asp Asp Leu Cys Asp Ala His
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225                 230                 235                 240

att ttc ctt ttc gaa cat ccc gaa gca aat ggt agg tac att tgt tct      768
Ile Phe Leu Phe Glu His Pro Glu Ala Asn Gly Arg Tyr Ile Cys Ser
                245                 250                 255

tca cat gat tcg aca att tat gac ttg gca aaa atg ctg aag aag aga      816
Ser His Asp Ser Thr Ile Tyr Asp Leu Ala Lys Met Leu Lys Lys Arg
            260                 265                 270

tat gcc aca tat gcc ata cct caa gag ttt aaa gat att gat cca aat      864
Tyr Ala Thr Tyr Ala Ile Pro Gln Glu Phe Lys Asp Ile Asp Pro Asn
        275                 280                 285

att aag aga gtg agt ttc tct tct aag aag ttc atg gac ttg ggg ttc      912
Ile Lys Arg Val Ser Phe Ser Ser Lys Lys Phe Met Asp Leu Gly Phe
    290                 295                 300

aag tac aag tac act att gag gag atg ttt gat gat gct att aag acc      960
Lys Tyr Lys Tyr Thr Ile Glu Glu Met Phe Asp Asp Ala Ile Lys Thr
305                 310                 315                 320

tgc agg gaa aag aat ctc tta ccg ccc aac act gag gaa cca gcc tta     1008
Cys Arg Glu Lys Asn Leu Leu Pro Pro Asn Thr Glu Glu Pro Ala Leu
                325                 330                 335

ctt gcc gaa aag tac gaa gaa atg aaa gaa caa ttg cag tta agt gaa     1056
Leu Ala Glu Lys Tyr Glu Glu Met Lys Glu Gln Leu Gln Leu Ser Glu
            340                 345                 350

aga aga atg aga agt ttg aaa att ctt tat gtt atc ctt tta ttt aca     1104
Arg Arg Met Arg Ser Leu Lys Ile Leu Tyr Val Ile Leu Leu Phe Thr
        355                 360                 365

cat ctg ctt tat tat gca tgg tta tat ctt gac tga                    1140
His Leu Leu Tyr Tyr Ala Trp Leu Tyr Leu Asp
    370                 375
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<210> 65
<211> 379
<212> PRT
<213> Doritaenopsis hybrid cultivar (Doritaenopsis Queen Beer)

<400> 65

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1               5                   10                  15
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            20                  25                  30

Val Arg Ala Thr Val Arg Asp Pro Thr Asn Ser Lys Lys Val Lys Pro
        35                  40                  45

Leu Leu Asp Leu Pro Gly Ser Asn Glu Leu Leu Ser Ile Trp Lys Ala
    50                  55                  60

Asp Leu Asn Asp Ile Glu Gly Ser Phe Asp Glu Val Ile Arg Gly Cys
65                  70                  75                  80

Val Gly Val Phe His Val Ala Thr Pro Met Asn Phe Gln Ser Lys Asp
                85                  90                  95

Pro Glu Asn Glu Val Ile Gln Pro Ala Ile Asn Gly Leu Leu Ser Ile
            100                 105                 110

Leu Arg Ser Cys Lys Arg Ser Gly Ser Val Arg Arg Val Ile Phe Thr
        115                 120                 125

Ser Ser Ala Gly Thr Val Asn Val Glu Glu Arg Arg Ala Pro Val Tyr
    130                 135                 140

Asp Glu Ser Ser Trp Ser Asp Leu Asp Phe Ile Thr Arg Val Lys Met
145                 150                 155                 160

Thr Gly Trp Met Tyr Phe Val Ser Lys Thr Leu Ala Glu Lys Ala Ala
                165                 170                 175

Trp Glu Phe Val Lys Glu Asn Asp Val Asp Phe Ile Ala Ile Ile Pro
            180                 185                 190

Thr Leu Val Val Gly Ser Phe Ile Thr Asp Glu Met Pro Pro Ser Leu
        195                 200                 205
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Thr Thr Ala Phe Ser Leu Ile Thr Gly Asn Glu Ala His Tyr Ser Ile
210 215 220

Ile Lys Gln Ala Gln Phe Val His Leu Asp Asp Leu Cys Asp Ala His
225 230 235 240

Ile Phe Leu Phe Glu His Pro Glu Ala Asn Gly Arg Tyr Ile Cys Ser
245 250 255

Ser His Asp Ser Thr Ile Tyr Asp Leu Ala Lys Met Leu Lys Lys Arg
260 265 270

Tyr Ala Thr Tyr Ala Ile Pro Gln Glu Phe Lys Asp Ile Asp Pro Asn
275 280 285

Ile Lys Arg Val Ser Phe Ser Ser Lys Lys Phe Met Asp Leu Gly Phe
290 295 300

Lys Tyr Lys Tyr Thr Ile Glu Glu Met Phe Asp Asp Ala Ile Lys Thr
305 310 315 320

Cys Arg Glu Lys Asn Leu Leu Pro Pro Asn Thr Glu Glu Pro Ala Leu
325 330 335

Leu Ala Glu Lys Tyr Glu Glu Met Lys Glu Gln Leu Gln Leu Ser Glu
340 345 350

Arg Arg Met Arg Ser Leu Lys Ile Leu Tyr Val Ile Leu Leu Phe Thr
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<212> DNA
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<220>
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<223> Primer PhANS5RACEGSP1

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<400> 71
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<400> 73
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<211> 1083
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<223> Doritaenopsis hybrid cultivar anthocyanidin synthase (PhANS 1)

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Asn Ser Gly Leu Ser Phe Ile Pro Ala Glu Phe Val Arg Pro Gln Ser
20 25 30

gaa cgc caa cac ctc caa gac tcc ctc aac aag aac ccc tgc ggt gtt 144
Glu Arg Gln His Leu Gln Asp Ser Leu Asn Lys Asn Pro Cys Gly Val
35 40 45

gag atc cca atc gtg gat ctc ggg ggg ttc tca tca gag gaa ggg cgg 192
Glu Ile Pro Ile Val Asp Leu Gly Gly Phe Ser Ser Glu Glu Gly Arg
50 55 60

cgg cgg cgg tgc gtg gag gag gtg atg gca gct gca gag gag tgg ggg 240
Arg Arg Arg Cys Val Glu Glu Val Met Ala Ala Ala Glu Glu Trp Gly
65 70 75 80

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Val Met Phe Leu Val Asn His Gly Val Pro Glu Glu Leu Ile Glu Arg
85 90 95

ctg cag gcg acg ggg aag ggg ttc ttc gaa ttg ccg gtg gac gag aag 336
Leu Gln Ala Thr Gly Lys Gly Phe Phe Glu Leu Pro Val Asp Glu Lys
100 105 110

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Glu Lys Tyr Ala Asn Asp Gln Ser Arg Gly Gln Ile Gln Gly Tyr Gly
        115                 120                 125

agc aag cta gca aat aat gaa aac ggt ata ctt gag tgg cag gat tac      432
Ser Lys Leu Ala Asn Asn Glu Asn Gly Ile Leu Glu Trp Gln Asp Tyr
    130                 135                 140

ttt ttt cac ctc gtc tac ccg ccg gag aag acg gac ctc acc atc tgg      480
Phe Phe His Leu Val Tyr Pro Pro Glu Lys Thr Asp Leu Thr Ile Trp
145                 150                 155                 160

ccg act gaa ccc gcg gac tac att gcg acc aca acc tcg ttc gcc aag      528
Pro Thr Glu Pro Ala Asp Tyr Ile Ala Thr Thr Thr Ser Phe Ala Lys
                165                 170                 175

gag ctc cga acc cta gcc tca aaa atg ttc tcc ata ctc tcc ctc ggt      576
Glu Leu Arg Thr Leu Ala Ser Lys Met Phe Ser Ile Leu Ser Leu Gly
            180                 185                 190

ctc ggc ctc gac caa aac aag ctc gaa gct gag ctc ggc ggc caa gac      624
Leu Gly Leu Asp Gln Asn Lys Leu Glu Ala Glu Leu Gly Gly Gln Asp
        195                 200                 205

gac ctc ctc ctc cag ctt aag atc aat tac tac ccg ccc tgc ccg cag      672
Asp Leu Leu Leu Gln Leu Lys Ile Asn Tyr Tyr Pro Pro Cys Pro Gln
    210                 215                 220

ccg gag ctg gcc ctc ggc gtc gag gcc cac acc gac gtc agc tcc ctc      720
Pro Glu Leu Ala Leu Gly Val Glu Ala His Thr Asp Val Ser Ser Leu
225                 230                 235                 240

tcc ttc atc ctt cac aac ggg atc ccc ggc ctc cag gtc ttc aag aac      768
Ser Phe Ile Leu His Asn Gly Ile Pro Gly Leu Gln Val Phe Lys Asn
                245                 250                 255

ggc gcc ggc tgg atc acc gct ccc ctc gtc cca aac tcg atc atc gtt      816
Gly Ala Gly Trp Ile Thr Ala Pro Leu Val Pro Asn Ser Ile Ile Val
            260                 265                 270

cac gtc ggg gat gcg ctc gag atc ctc agc aat ggg agg tgc cac agc      864
His Val Gly Asp Ala Leu Glu Ile Leu Ser Asn Gly Arg Cys His Ser
        275                 280                 285

gtt ctt cac cga gga ctt gtt act aag gaa aat gtt cgg atc tcg tgg      912
Val Leu His Arg Gly Leu Val Thr Lys Glu Asn Val Arg Ile Ser Trp
    290                 295                 300
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305 310 315 320

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Leu Glu Leu Ile Gly Lys Gly Glu Val Ala Arg Phe Glu Pro Arg Thr
325 330 335

ttt gcg gag cat ttg gag agg aag ctg ttc aag ccg agg gtg gag ggt 1056
Phe Ala Glu His Leu Glu Arg Lys Leu Phe Lys Pro Arg Val Glu Gly
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Cys Gly Glu Lys Ala Pro Val Asp
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<211> 360
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<400> 75

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20 25 30

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35 40 45

Glu Ile Pro Ile Val Asp Leu Gly Gly Phe Ser Ser Glu Glu Gly Arg
50 55 60

Arg Arg Arg Cys Val Glu Glu Val Met Ala Ala Ala Glu Glu Trp Gly
65 70 75 80

Val Met Phe Leu Val Asn His Gly Val Pro Glu Glu Leu Ile Glu Arg
85 90 95

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Leu Gln Ala Thr Gly Lys Gly Phe Phe Glu Leu Pro Val Asp Glu Lys
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Glu Lys Tyr Ala Asn Asp Gln Ser Arg Gly Gln Ile Gln Gly Tyr Gly
        115                 120                 125

Ser Lys Leu Ala Asn Asn Glu Asn Gly Ile Leu Glu Trp Gln Asp Tyr
    130                 135                 140

Phe Phe His Leu Val Tyr Pro Pro Glu Lys Thr Asp Leu Thr Ile Trp
145                 150                 155                 160

Pro Thr Glu Pro Ala Asp Tyr Ile Ala Thr Thr Thr Ser Phe Ala Lys
                165                 170                 175

Glu Leu Arg Thr Leu Ala Ser Lys Met Phe Ser Ile Leu Ser Leu Gly
            180                 185                 190

Leu Gly Leu Asp Gln Asn Lys Leu Glu Ala Glu Leu Gly Gly Gln Asp
        195                 200                 205

Asp Leu Leu Leu Gln Leu Lys Ile Asn Tyr Tyr Pro Pro Cys Pro Gln
    210                 215                 220

Pro Glu Leu Ala Leu Gly Val Glu Ala His Thr Asp Val Ser Ser Leu
225                 230                 235                 240

Ser Phe Ile Leu His Asn Gly Ile Pro Gly Leu Gln Val Phe Lys Asn
                245                 250                 255

Gly Ala Gly Trp Ile Thr Ala Pro Leu Val Pro Asn Ser Ile Ile Val
            260                 265                 270

His Val Gly Asp Ala Leu Glu Ile Leu Ser Asn Gly Arg Cys His Ser
        275                 280                 285
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Val Leu His Arg Gly Leu Val Thr Lys Glu Asn Val Arg Ile Ser Trp
290 295 300

Ala Val Phe Cys Glu Pro Pro Arg Glu Lys Val Val Leu Arg Pro Leu
305 310 315 320

Leu Glu Leu Ile Gly Lys Gly Glu Val Ala Arg Phe Glu Pro Arg Thr
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Cys Gly Glu Lys Ala Pro Val Asp
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<210> 84
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<212> DNA
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<223> Primer PhCHS3-1073R

<400> 87
cgagtatgaa aagaacgcaa gcgctcgaca tgttac 36

Claims

1. A gene encoding a flavonoid 3',5'-hydroxylase of *Commelina communis,* which comprises an amino acid sequence depicted in SEQ ID No: 2 or an amino acid sequence having at least 90% homology to the amino acid sequence depicted in SEQ ID No: 2.

2. A vector, which contains the gene as defined in Claim 1.

3. A method for producing a flower color-changed plant, which comprises transfecting a plant with the gene as defined in Claim 1 and expressing the gene.

4. The method for producing a flower color-changed plant according to Claim 3, wherein the plant is an *Orchid* family plant.

5. The method for producing a flower color-changed plant according to Claim 3, wherein the plant is moth orchid or cymbidium.

6. A method for producing a flower color-changed plant, which comprises transfecting an Orchid family plant with the gene as defined in Claim 1 and a gene encoding a dihydroflavonol 4-reductase of Torenia or Gerbera and expressing the genes.

7. The method for producing a flower color-changed plant according to Claim 6, wherein the Orchid family plant is moth orchid or cymbidium.

8. A method for producing an Orchid family plant having a blue flower, which comprises transfecting an Orchid family plant having a white flower with the gene as defined in Claim 1; a gene encoding a dihydroflavonol 4-reductase of *Torenia* or *Gerbera*; a gene encoding a flavanone 3-hydroxylase; and a gene encoding an anthocyanidin synthase and expressing the genes.

9. The method for producing an Orchid family plant having a blue flower according to Claim 8, wherein the Orchid family plant is moth orchid or cymbidium.

10. A flower color-changed plant, which is produced by the method as defined in Claim 3, 6 or 8, wherein the gene encoding a flavanone 3-hydroxylase is derived from moth orchid, and the gene encoding an anthocyanidin synthase is derived from moth orchid, *Torenia* or *Gerbera*; a progeny having the same characters as the changed flower color; or its tissue.

11. A flower color-changed plant having the gene as defined in Claim 1.

12. The flower color-changed plant according to Claim 11, which is an *Orchid* family plant.

13. The flower color-changed plant according to Claim 12, which is moth orchid or cymbidium.

Fig.1

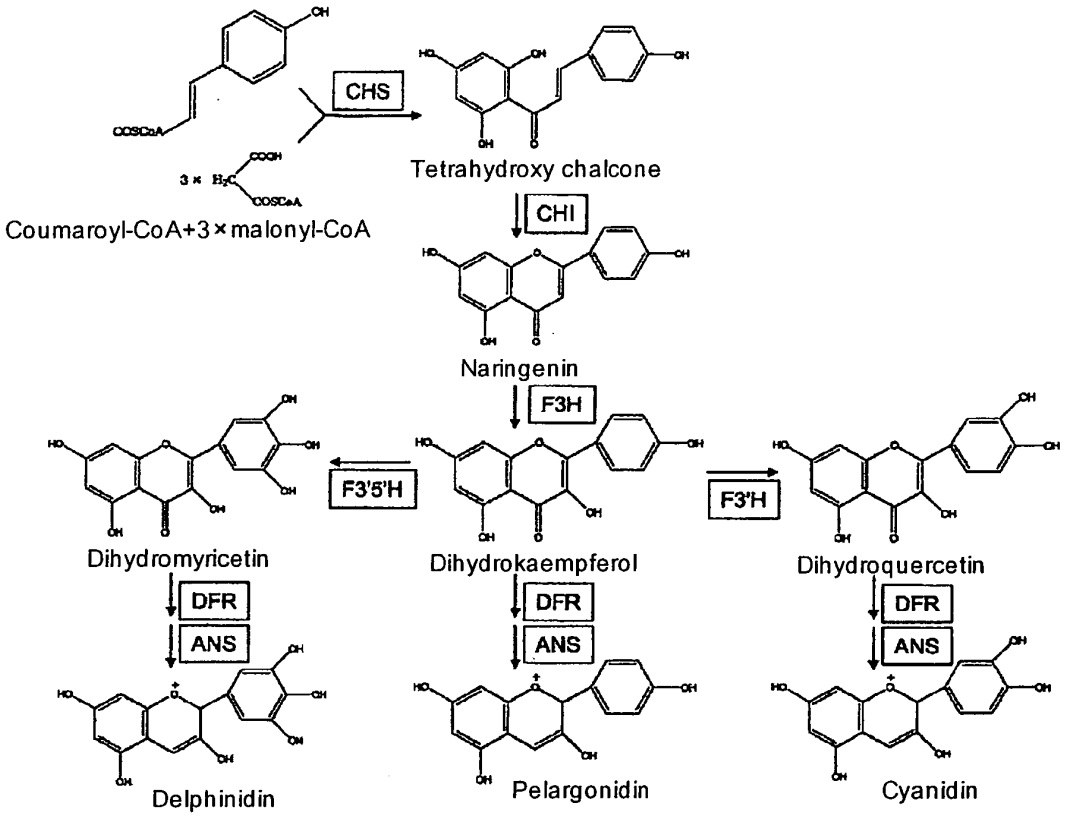

CHS
Coumaroyl-CoA+3×malonyl-CoA
Tetrahydroxy chalcone
CHI
Naringenin
F3H
F3'5'H
F3'H
Dihydromyricetin
Dihydrokaempferol
Dihydroquercetin
DFR
ANS
DFR
ANS
DFR
ANS
Delphinidin
Pelargonidin
Cyanidin

Fig.2

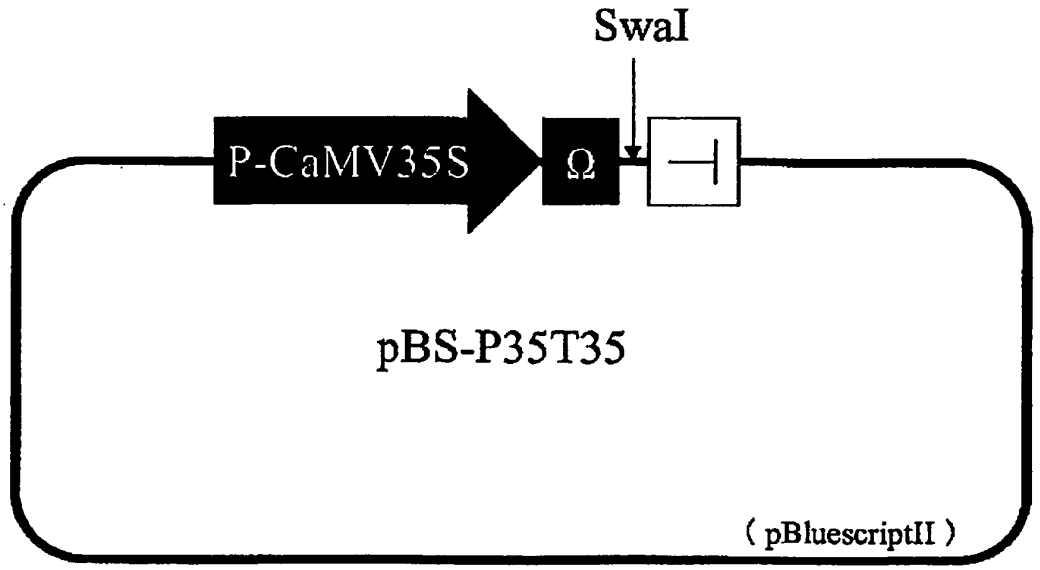

SwaI
P-CaMV35S
Ω
pBS-P35T35
(pBluescriptII)

Fig.3

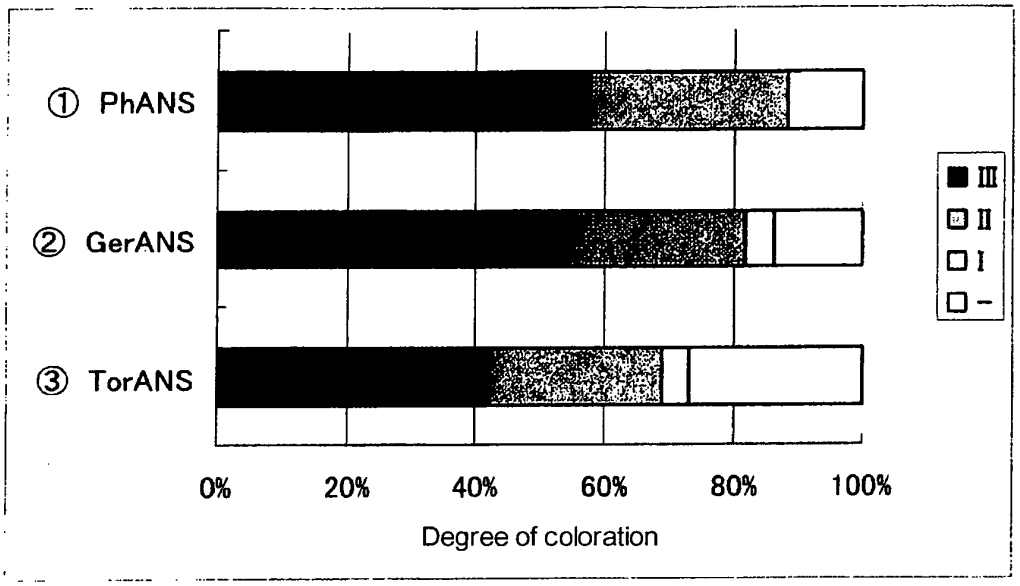

① PhANS
② GerANS
③ TorANS
III
II
I
–
0%
20%
40%
60%
80%
100%
Degree of coloration

Fig.4

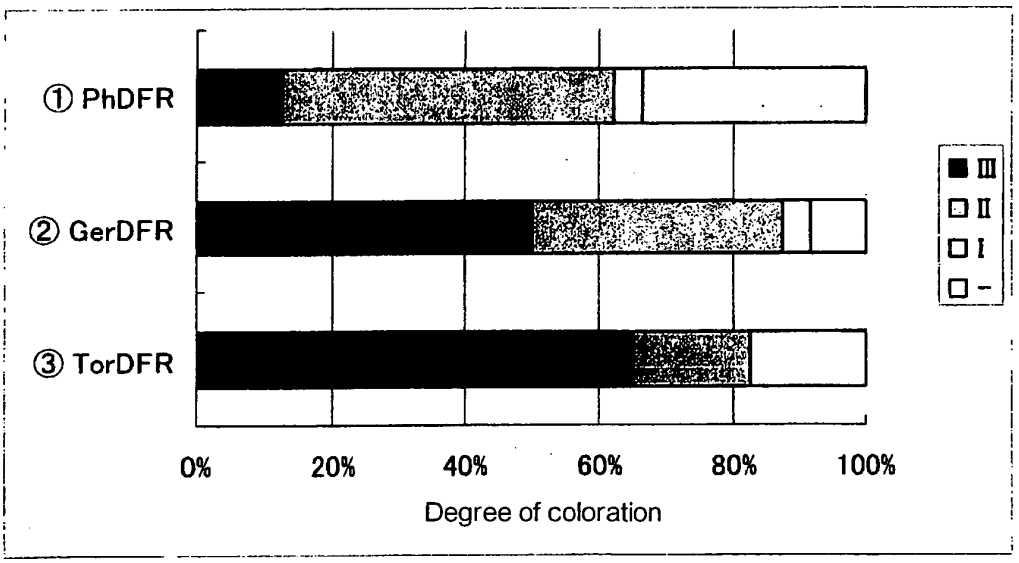

① PhDFR
② GerDFR
③ TorDFR
III
II
I
–
0%
20%
40%
60%
80%
100%
Degree of coloration

Fig.5

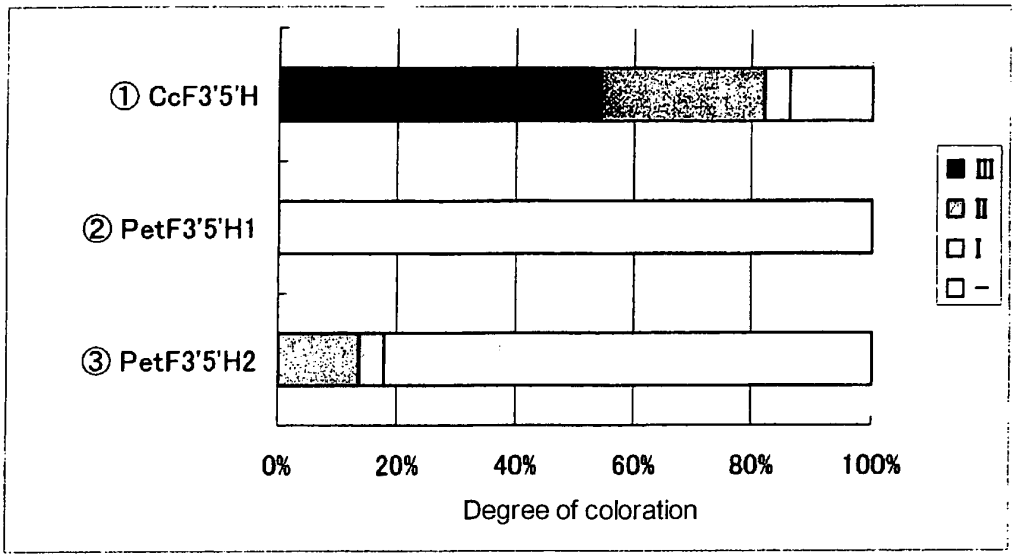

① CcF3'5'H
② PetF3'5'H1
③ PetF3'5'H2
III
II
I
–
0%
20%
40%
60%
80%
100%
Degree of coloration

Fig.6

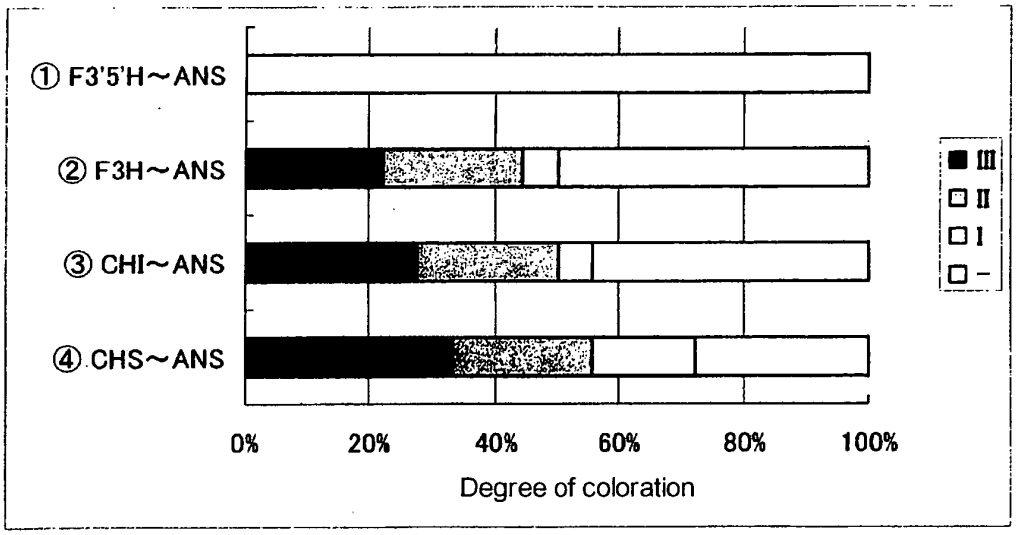

① F3'5'H～ANS
② F3H～ANS
③ CHI～ANS
④ CHS～ANS
III
II
I
–
0%
20%
40%
60%
80%
100%
Degree of coloration

Fig.7

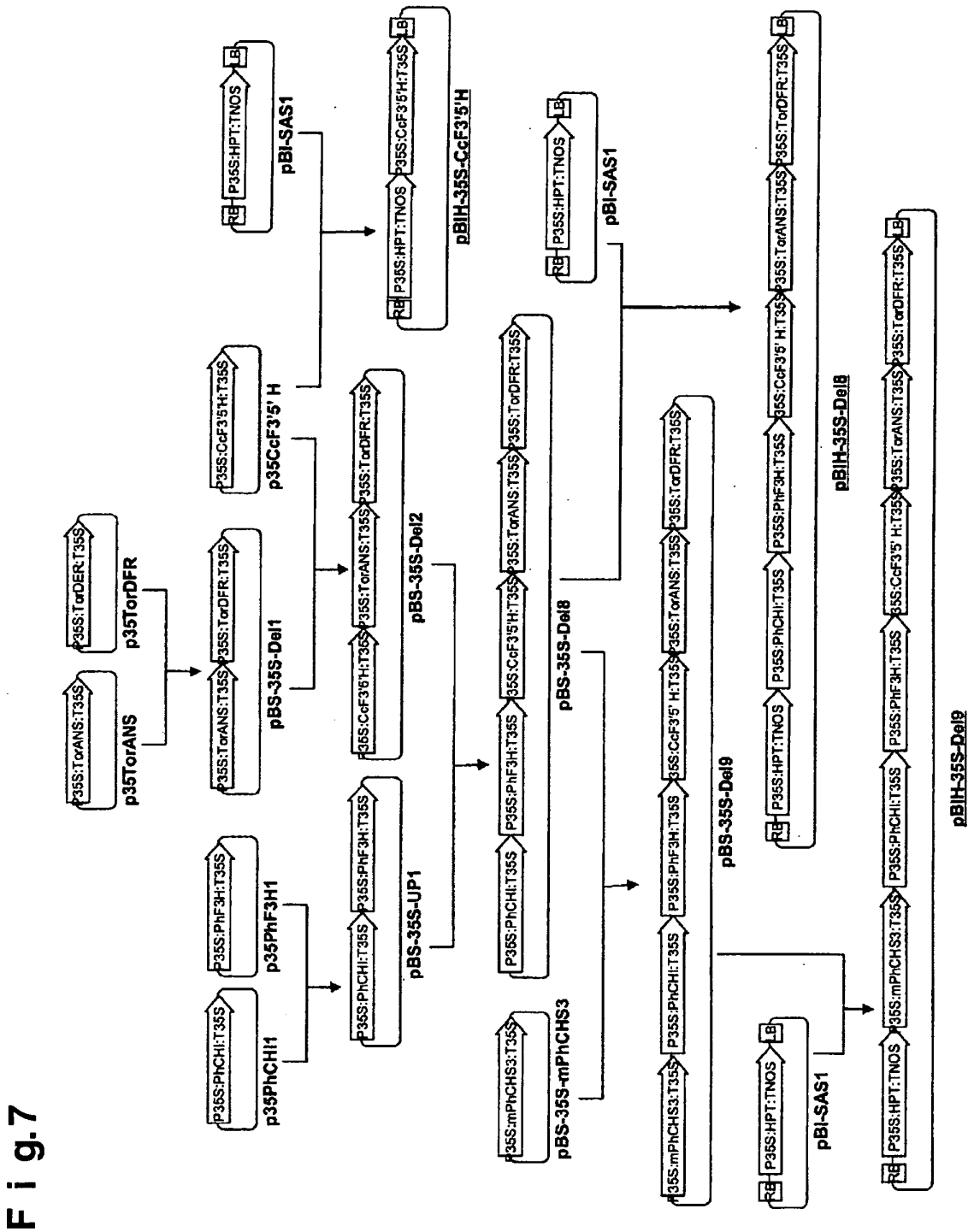

p35TorANS
p35TorDFR
p35PhCHI1
p35PhF3H1
pBS-35S-Del1
p35CcF3'5' H
pBI-SAS1
pBIH-35S-CcF3'5'H
pBS-35S-UP1
pBS-35S-Del2
pBS-35S-mPhCHS3
pBS-35S-Del8
pBS-35S-Del9
pBIH-35S-Del8
pBIH-35S-Del9

Fig.8

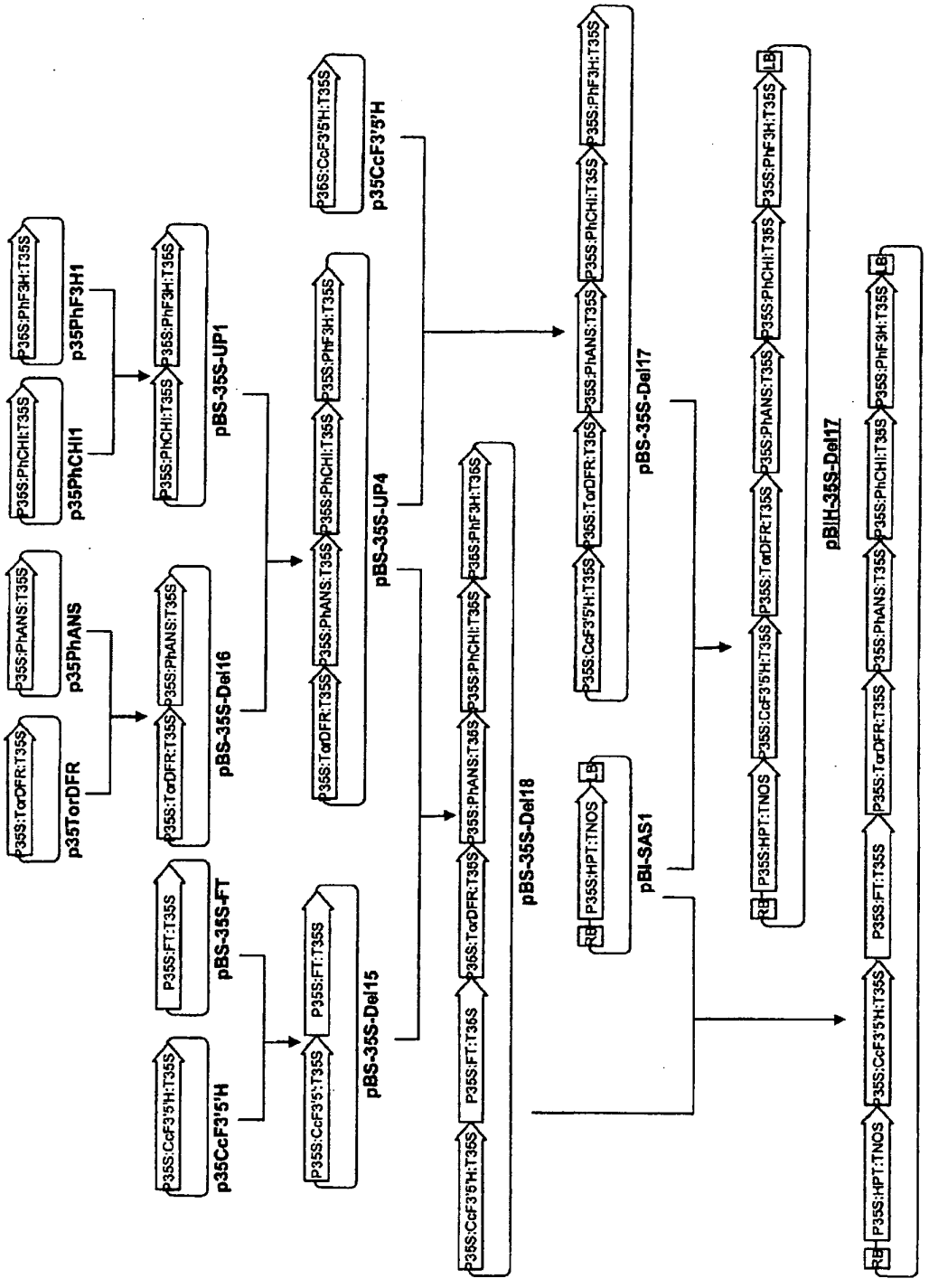
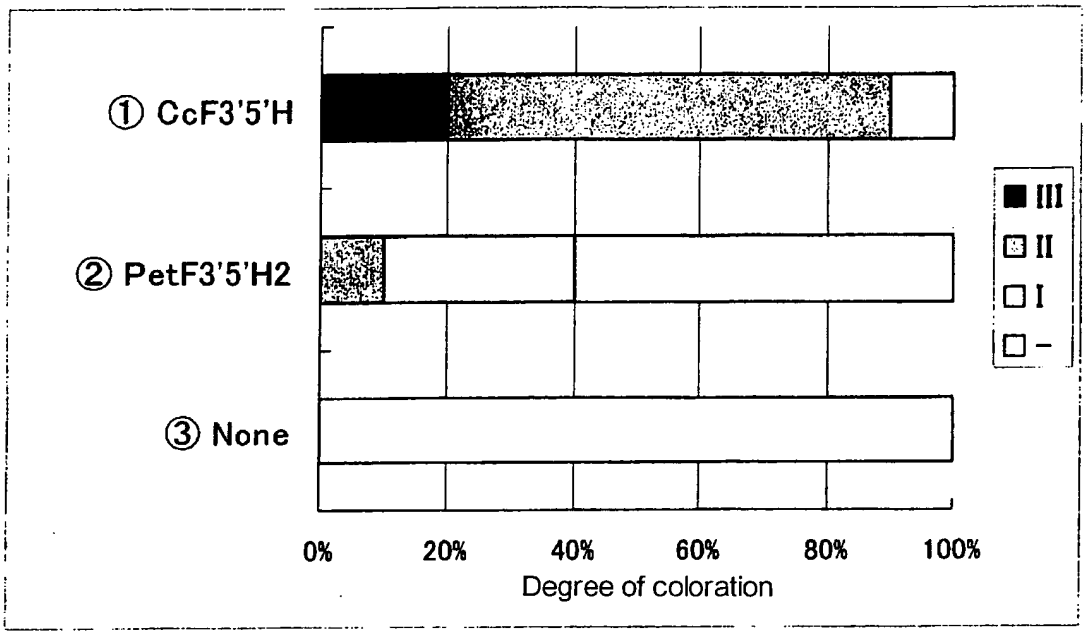

P35S:TorDFR:T35S
p35TorDFR
P35S:PhANS:T35S
p35PhANS
P35S:PhCHI:T35S
p35PhCHI
P35S:PhF3H:T35S
p35PhF3H
P35S:CcF3'5'H:T35S
p35CcF3'5'H
P35S:FT:T35S
pBS-35S-FT
pBS-35S-Del16
pBS-35S-UP1
pBS-35S-Del15
pBS-35S-UP4
p35CcF3'5'H
pBS-35S-Del18
RB
P35S:HPT:TNOS
LB
pBI-SAS1
pBS-35S-Del17
pBIH-35S-Del17
pBIH-35S-Del18

Fig.9


① CcF3'5'H
② PetF3'5'H2
③ None
III
II
I
–
0%
20%
40%
60%
80%
100%
Degree of coloration

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2008/054653

A. CLASSIFICATION OF SUBJECT MATTER
C12N15/00(2006.01)i, *A01H1/00*(2006.01)i, *A01H5/00*(2006.01)i, *C12N15/09*(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N15/00-15/90, A01H1/00, A01H5/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2008
Kokai Jitsuyo Shinan Koho 1971-2008 Toroku Jitsuyo Shinan Koho 1994-2008

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS/MEDLINE/WPIDS(STN), JSTPlus(JDreamII), JMEDPlus(JDreamII), GenBank/EMBL/DDBJ/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2006-512057 A (INTERNATIONAL FLOWER DEVELOPMENTS Proprietary Ltd.), 13 April, 2006 (13.04.06), Full text (Family: none)	1-5,11-13
Y	SU,V. & HSU,B-D "Cloning and expression of a putative cytochrome P450 gene that influences the color of Phalaenopsis flowers." Biotechnol.Lett., 2003, Vol.25, p.1933-1939	1-7,10-13
Y	EP 1652916 A1 (INTERNATIONAL FLOWER DEVELOPMENTS Proprietary Ltd.), 03 May, 2006 (03.05.06), Full text & WO 2005/017147 A1 & CA 002537065 A & CN 001836039 A	1-7,10-13

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 May, 2008 (08.05.08)	20 May, 2008 (20.05.08)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2008/054653

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NAKATSUKA, T., et al., "Temporal expression of flavonoid biosynthesis-related genes regulates flower pigmentation in gentian plants." Plant Sci., 2005, Vol.168, p.1309-1318	1-7,10-13
Y	SEITZ, C., et al., "Cloning, functional identification and sequence analysis of flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase cDNAs reveals independentevolution of flavonoid 3',5'-hydroxylase in the Asteraceae family." Plant Mol.Biol., 2006, Vol.61, p.365-381	1-7,10-13
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REFERENCES CITED IN THE DESCRIPTION

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- **Mishiba et al.** *Plant Cell Reports,* 2005, vol. 24, 297-303 **[0113]**
- **Kobayashi et al.** *Science,* 1999, vol. 286, 1960-1962 **[0115]**