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(54) Titre : GENES DE SYNTHÈSE DE RAFFINOSE ET LEUR UTILISATION

(54) Title: RAFFINOSE SYNTHASE GENES AND THEIR USE

(57) **Abrégé/Abstract:**

A raffinose synthase gene comprising a nucleotide sequence hybridizable with a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, (b) the nucleotide sequence represented by SEQ ID NO: 2, (c) a nucleotide sequence encoding the amino acid sequence of represented by SEQ ID NO: 3, (d) the nucleotide sequence represented by SEQ ID NO: 4 or by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4, (e) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5, (f) the nucleotide sequence represented by SEQ ID NO: 6 or by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6, (g) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, and (h) the nucleotide sequence represented by SEQ ID NO: 8 or by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8, under stringent conditions, and encoding a protein being capable of binding D-galactosyl group through α (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose is disclosed.



Abstract of the disclosure:

A raffinose synthase gene comprising a nucleotide sequence hybridizable with a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, (b) the nucleotide sequence represented by SEQ ID NO: 2, (c) a nucleotide sequence encoding the amino acid sequence of represented by SEQ ID NO: 3, (d) the nucleotide sequence represented by SEQ ID NO: 4 or by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4, (e) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5, (f) the nucleotide sequence represented by SEQ ID NO: 6 or by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6, (g) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, and (h) the nucleotide sequence represented by SEQ ID NO: 8 or by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8, under stringent conditions, and encoding a protein being capable of binding D-galactosyl group through α (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose is disclosed.

RAFFINOSE SYNTHASE GENES AND THEIR USE

BACKGROUND OF THE INVENTION

5 FIELD OF INVENTION

The present invention relates to raffinose synthase genes and their use.

DISCLOSURE OF THE RELATED ART

10 Raffinose family oligosaccharides are derivatives of sucrose, which are represented by the general formula: o- α -D-galactopyranosyl-(1 $\rightarrow$ 6)_n-o- α -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fluctofuranoside, and they are called "raffinose" when n is 1, "stachyose" when n is 2, "verbascose" when n is 3, and "ajugose" when n is 4.

15 It has been known that raffinose family oligosaccharides have an effect of giving good conditions of enterobacterial flora, if present at an appropriate amount in food. Therefore, raffinose family oligosaccharides have already been used as a functional food material for addition
20 to some kinds of food and utilized in the field of specific health food. On the other hand, raffinose family oligosaccharides are neither digested nor absorbed in mammals such as human, but are assimilated and decomposed by enterobacteria to generate gases and to cause meteorism and absorption disorder. Therefore, it
25 has been desired to appropriately regulate the amount of raffinose family oligosaccharides in food and feed.

- 2 -

Raffinose family oligosaccharides are synthesized by the raffinose family oligosaccharide biosynthesis system beginning with sucrose in many plants. This biosynthesis system normally involves a reaction for the sequential addition of galactosyl groups from galactinol through an α (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule. Raffinose synthase is the enzyme concerned in the reaction for producing raffinose by allowing a D-galactosyl group derived from galactinol to form the α (1 $\rightarrow$ 6) bond with the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule in the first step of this biosynthesis system. It has been suggested that this enzyme constitutes a rate-limiting step in the above synthesis system, and therefore this enzyme is quite important in the control of biosynthesis of raffinose family oligosaccharides.

Then, a method for controlling an expression level or activity of raffinose synthase in plants by utilizing a raffinose synthase gene is effective to control a biosynthesis system of raffinose family oligosaccharides in plants to increase or decrease the production of raffinose in plants.

Thus, a raffinose synthase gene which can be used in such a method has been desired.

- 3 -

The main object of the present invention is to provide novel raffinose synthase genes from plants.

This object as well as other objects and advantage of the present invention will become apparent to those skilled
5 in the art from the following description.

SUMMARY OF THE INVENTION

Under these circumstances, the present inventors have intensively studied and succeeded in isolating novel genes
10 encoding raffinose synthase from various plants. Thus, the present invention has been completed.

That is, the present invention provides:

1. A raffinose synthase gene which comprises a nucleotide sequence hybridizable with a nucleotide sequence
15 selected from the group consisting of:

(a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1,

(b) the nucleotide sequence represented by SEQ ID NO:
2,

20 (c) a nucleotide sequence encoding the amino acid sequence of represented by SEQ ID NO: 3,

(d) the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4,

25 (e) a nucleotide sequence encoding the amino acid

- 4 -

sequence represented by SEQ ID NO: 5,

(f) the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6,

5 (g) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, and

(h) the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8,

10 under stringent conditions, and encoding a protein being capable of binding D-galactosyl group through α (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose.

2. A raffinose synthase gene comprising a
15 nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1.

3. A raffinose synthase gene comprising the nucleotide sequence represented by SEQ ID NO: 2.

4. A raffinose synthase gene comprising a
20 nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 3.

5. A raffinose synthase gene comprising the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID
25 NO: 4.

- 5 -

6. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5.

5 7. A raffinose synthase gene comprising the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6.

8. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence
10 represented by SEQ ID NO: 7.

9. A raffinose synthase gene comprising the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8.

10. A raffinose synthase gene comprising the
15 nucleotide sequence represented by SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

11. A nucleic acid comprising a partial nucleotide sequence of the raffinose synthase gene of any one of the above 1 to 10.

20 12. A method for detecting a nucleic acid containing a raffinose synthase gene which comprises detecting said nucleic acid by hybridization using the labeled nucleic acid of the above 11 as a probe.

13. A method for amplifying a nucleic acid
25 containing a raffinose synthase gene which comprises amplifying

- 6 -

said nucleic acid by polymerase chain reaction (PCR) using the nucleic acid of the above 11 as a primer.

14. A method for obtaining a raffinose synthase gene which comprises the steps of:

5 detecting a nucleic acid containing said raffinose synthase gene by hybridization using the labeled nucleic acid of the above 11 as a probe, and

 recovering the detected nucleic acid.

15 15. A method for obtaining a raffinose synthase gene which comprises the steps of:

 amplifying a nucleic acid containing said raffinose synthase gene by PCR using the nucleic acid of the above 11 as a primer, and

 recovering the amplified nucleic acid.

15 16. A nucleic acid comprising a nucleic acid containing the raffinose synthase gene of any one of the above 1 to 10 which is joined to a nucleic acid exhibiting promoter activity in a host cell.

20 17. A vector comprising the raffinose synthase gene of any one of the above 1 to 10.

 18. A transformant, wherein the raffinose synthase gene of any one of the above 1 to 10 is introduced into a host cell.

25 19. A transformant, wherein the nucleic acid of the above 16 is introduced into a host cell.

- 7 -

20. A transformant, wherein the vector of the above 17 is introduced into a host cell.

21. The transformant of any one of the above 18 to 20, wherein the host is a microorganism.

5 22. The transformant of any one of the above 18 to 20, wherein the host is a plant.

23. A method for producing a raffinose synthase which comprises the steps of:

10 culturing or growing the transformant of any one of the above 18 to 22 to produce the raffinose synthase, and collecting the raffinose synthase.

24. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 1.

15 25. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 3.

26. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 5.

27. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 7.

20 The term "nucleic acid" used herein means an oligomer compound or a high molecular compound which is generally called "DNA" or "RNA".

DETAILED DESCRIPTION OF THE INVENTION

25 The gene engineering techniques described below can

- 8 -

be carried out, for example, according to methods described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press, ISBN 0-87969-309-6; "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X; "Current Protocols In Protein Science" (1995), John Wiley & Sons, Inc. ISBN 0-471-11184-8.

The genes of the present invention can be obtained from soybean, plants belonging to the families Chenopdiaceae such as beet, etc. and Cruciferae such as mustard, rapeseed, etc. Specific examples of the genes of the present invention include those comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, the nucleotide sequence represented by SEQ ID NO: 2, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 3, the nucleotide sequence represented by SEQ ID NO: 4 or by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5, the nucleotide sequence represented by SEQ ID NO: 6 or by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6, a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, the nucleotide sequence represented by SEQ ID NO: 8 or by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8, and the like.

The genes of the present invention can be obtained,

- 9 -

for example, by the following method.

That is, the genes of the present invention derived from soybean can be obtained, for example, by the following method.

5 For example, the gene can be obtained by a hybridization method using a nucleic acid having the nucleotide sequence represented by SEQ ID NO: 2 or its partial nucleotide sequence as a probe to detect a nucleic acid fragment which hybridizes to the probe in DNAs derived from soybean, followed
10 by isolating the detected nucleic acid.

In this method, first, a nucleic acid to be used as the probe is prepared. As such a nucleic acid, for example, there is a nucleic acid composed of an oligonucleotide chemically synthesized by a conventional method on the basis of the
15 nucleotide sequence of SEQ ID NO: 2. Specific example thereof includes a nucleic acid having the 800th to the 899th nucleotides in the nucleotide sequence represented by SEQ ID NO: 2.

Alternatively, the gene of the present invention derived from soybean can be obtained by the following method.

20 For example, tissue of soybean (*Glycine max*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in
25 the extraction. RNA is recovered from thus-obtained RNA extract

- 10 -

by ethanol precipitation. Poly-A tailed RNA is fractionated from thus-recovered RNA by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation. cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 2. More specifically, as the primers, for example, there are primers 11 and 12 shown in List 1 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from soybean, the genes of the present invention derived from soybean, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1" and the "raffinose synthase gene having the nucleotide sequence of SEQ ID No: 2" can be obtained.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X. Alternatively, cloning can be carried out, for example, by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such

- 11 -

as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 1

Primer 11: ccaatctgat catgcttggtg ccgaa 25mer
 10 Primer 12: ggaacaaagt tatgcactat tattttaaggt 30mer

The genes of the present invention derived from a Chenopdiaceae plant such as beet can be obtained by the following method.

For example, tissue of a Chenopdiaceae plant such as beet (*Beta vulgaris*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From this tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in the extraction. RNA is recovered from the thus-obtained RNA extract by ethanol precipitation. From the recovered RNA, poly-A tailed RNA is fractionated by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation.

cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by

- 12 -

utilizing a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. More specifically, as the primers, for example, there are primers 21 and 22 shown in List 2 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from beet, the genes of the present invention derived from beet, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3," and the "raffinose synthase gene having a nucleotide sequence of SEQ ID No: 4" can be obtained.

According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. For example, in order to amplify the "raffinose synthase gene having the nucleotide sequence represented by the 236th to the 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4", preferably, oligonucleotides having the nucleotide sequences represented by primers 23 and 24 in List 2 below are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

- 13 -

Alternatively, cloning can be carried out by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined, for example, by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 2

Primer 21:	ctaccaaatt ccacaactta aagttca	27mer
Primer 22:	ggaataataa gcttcacaca tactgtactc tc	32mer
15 Primer 23:	atggctccaa gcttttagcaa ggaaaattcc	30mer
Primer 24:	tcaaaataag tactcaacag tggtaaaacc	30mer

The genes of the present invention derived from Cruciferae plants such as mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) can be obtained by the following method.

20 For example, tissue of a Cruciferae plant such as mustard or rapeseed is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available

- 14 -

RNA extraction kit can be utilized in the extraction. The RNA is recovered from thus-obtained RNA extract by ethanol precipitation. Poly-A tailed RNA is fractionated from the RNA thus recovered by a conventional method. A commercially

5 available oligo-dT column can be utilized in the fractionation.

cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA are amplified by PCR using the above-obtained cDNA as a template

10 and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, when PCR is carried out by using cDNA derived from mustard (*Brassica juncea*) as the template and primers 33 and 34 shown in List 3 hereinafter, the genes from Cruciferae plants of the present

15 invention, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 5," and the "raffinose synthase gene having the nucleotide sequence represented by the 1st to 2654th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6" can be obtained.

20 According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, in order to amplify DNA encoding the open reading frame region of the "raffinose synthase gene having a nucleotide sequence encoding a protein having the

- 15 -

amino acid sequence of SEQ ID NO: 5", and the "raffinose synthase gene having the nucleotide sequence represented by the 134th to the 2467th nucleotides of SEQ ID NO: 6", preferably, oligonucleotides having the nucleotide sequences represented by primers 35 and 36 in List 3 are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

Alternatively, cloning can be carried out, for example, by using a commercially available TA cloning kit (Invitrogen) or a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, the commercially available ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer can be used.

List 3

Primer 31: ttggaagaga agacgccgcc gggaatcgtc 30mer
Primer 32: ttaagccccg gcgagagctc tggccggaca 30mer
Primer 33: accaatccaa aatctcatca aataatcgca 30mer

- 16 -

Primer 34: aaataatagg ggcagtacaa attacaccac 30mer

Primer 35: atggctccac cgagcgtaat taaatccga 29mer

Primer 36: ctaaaactca tacttaatag aagacaaacc 30mer

Then, a nucleic acid having a partial nucleotide
5 sequence of the gene of the present invention (hereinafter
referred to as "the gene fragment") which is obtained by the
above-described method is labeled and then used as a probe in
a hybridization method. The probe can be hybridized to, for
example, DNA derived from soybean, a Chenopdiaceae plant or a
10 Cruciferae plant to detect a nucleic acid having the probe
specifically bound thereto, thereby detecting a nucleic acid
having the raffinose synthase gene.

As the DNA derived from soybean, a Chenopdiaceae plant
such as beet or a Cruciferae plant such as mustard or rapeseed,
15 for example, a cDNA library or a genomic DNA library of these
plants can be used. The gene library may also be a commercially
available gene library as such or a library constructed according
to a conventional library construction method, for example,
described in "Molecular Cloning: A Laboratory Manual 2nd
20 edition" (1989), Cold Spring Harbor Laboratory Press; "Current
Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc.
ISBN 0-471-50338-X.

As the hybridization method, for example, plaque
hybridization or colony hybridization can be employed, and they
25 are selected depending upon the kind of vector used in the

- 17 -

construction of a library. More specifically, when the library to be used is constructed with a phage vector, a suitable host microorganism is mixed with the phage of the library under infectious conditions to obtain transformants. The transformant is further mixed with a soft agar medium, and the mixture is plated on an agar medium. Thereafter, the mixture is cultured at 37°C until a plaque of an appropriate size appears. When the library to be used is constructed with a plasmid vector, the plasmid is introduced into a suitable host microorganism to form transformants. The transformant obtained is diluted to a suitable concentration and the dilution is plated on an agar medium, after which it is cultured at 37°C until a colony of an appropriate size appears. In either case of the above libraries, a membrane filter is placed on the surface of the agar medium after the above cultivation, so that the phage or transformant is transferred to the membrane. This membrane is denatured with an alkali, followed by neutralization, and for example, when a nylon membrane is used, the membrane is irradiated with ultraviolet light, so that DNA of the phage or transformant is fixed on the membrane. This membrane is then subjected to a hybridization method wherein the gene fragment which has a partial nucleotide sequence of the gene of the present invention and labeled by a conventional method (hereinafter referred to as "the labeled gene fragment") is used as a probe. For this method, reference may be made, for example, to D.M.

- 18 -

Glover ed., "DNA cloning, a practical approach" IRL PRESS (1985), ISBN 0-947946-18-7. There are various reagents and temperature conditions to be used in the hybridization. For example, in general, prehybridization is carried out by immersion of the
5 membrane in a prehybridization solution [6 x SSC (0.9 M NaCl, 0.09 M citric acid), 0.1 to 1 (w/v)% SDS, 100 µg/ml denatured salmon sperm DNA] and incubation at 65°C for 1 hour. Then, hybridization is carried out by addition and mixing of the labeled gene fragment thereto and incubating the membrane at
10 42 to 68°C for 4 to 16 hours.

In the present invention, the "stringent conditions" are those wherein incubation is carried out, for example, at 65 to 68°C in the above hybridization.

After hybridization, the membrane is taken out and
15 is washed with 2 x SSC containing 0.1 to 1 (w/v)% SDS, further rinsed with 0.2 x SSC containing 0.1 to 1 (w/v)% SDS, and then dried. The membrane is analyzed, for example, by autoradiography or other techniques to detect the position of the probe on the membrane, thereby detecting the position on
20 the membrane of a nucleic acid having a nucleotide sequence homologous to that of the probe used. The clone corresponding to the position of the nucleic acid thus detected on the membrane is identified on the original agar medium and the positive clone is selected so that the clone having the nucleic acid can be

- 19 -

isolated. The same procedures of detection are repeated to purify the clone having the nucleic acid.

Alternatively, a commercially available kit such as GENE TRAPPER cDNA Positive Selection System kit (GibcoBRL) can be used. In this method, first, a single-stranded DNA library is hybridized with the biotinylated gene fragment (i.e., probe), followed by adding streptoavidin-bound magnet beads and mixing.

From the mixture, the streptoavidin-bound magnetic beads are collected with a magnet, so that single-stranded DNA having a nucleotide sequence homologous to that of the probe used, which has been bound to these beads through the gene fragment, biotin and streptoavidin, is collected and detected. The single-stranded DNA collected can be converted into a double-strand form by reaction with a suitable DNA polymerase using a suitable oligonucleotide as a primer.

As described above, a nucleic acid containing raffinose synthase gene can be obtained by detecting a nucleic acid hybridizable to the gene fragment in DNAs of a gene library derived from soybean, a Chenopodiaceae plant or a Cruciferae plant, purifying a clone having the nucleic acid and isolating phage or plasmid DNA from the clone. By preparing the restriction map or determining the nucleotide sequence of the nucleic acid thus obtained according to a conventional method, the nucleic acid containing the gene of the present invention can be confirmed.

For example, the gene of the present invention from

- 20 -

a Chenopdiaceae plant can be confirmed by the following point:

5 The amino acid encoded by the nucleotide sequence thus determined has 75% or more homology to the amino acid sequence represented by the 103rd to 208th amino acids in the amino acid sequence of SEQ ID NO: 3;

80% or more homology to the amino acid sequence represented by the 255th to 271st amino acids in the amino acid sequence of SEQ ID NO:3;

10 70% or more homology to the amino acid sequence represented by the 289th to 326th amino acids in the amino acid sequence of SEQ ID NO: 3; or

70% or more homology to the amino acid sequence represented by the 610th to 696th amino acids in the amino acid sequence of SEQ ID NO: 3.

15 The gene of the present invention from a Cruciferae plant can be confirmed, for example, by the following point:

20 The amino acid sequence encoded by the nucleotide sequence determined has 75% or more homology to the amino acid sequence represented by the 111th to 213th amino acids in the amino acid sequence of SEQ ID NO: 5;

80% or more homology to the amino acid sequence represented by the 260th to 275th amino acids in the amino acid sequence of SEQ ID NO: 5;

25 70% or more homology to the amino acid sequence represented by the 293rd to 325th amino acids in the amino acid

- 21 -

sequence of SEQ ID NO: 5; or

70% or more homology to the amino acid sequence represented by the 609th to 695th amino acids in the amino acid sequence of SEQ ID NO: 5.

5 The "homology" used herein means the proportion of the number of amino acids in a region, which are identical to those in a different region to be compared, to the number of the entire amino acids in the former region, upon comparing regions having similarity in two amino acid sequences. In this
10 respect, it is preferred that the region having similarity contains more amino acids. Such homology of amino acid sequences can be evaluated by using a commercially available gene analysis software such as GENETIX (Software Kaihatu K.K.).

 Further, according to the same manner as described
15 above, a nucleic acid containing raffinose synthase gene can be detected by hybridization to DNA from the desired organism using the gene fragment as a probe to detect a nucleic acid to which the probe specifically binds (hereinafter referred to as the detection method of the present invention). The gene
20 fragment used herein can be chemically synthesized according to a conventional method on the basis of the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or 8. Alternatively, it can be prepared by PCR using as primers oligonucleotides chemically
25 synthesized according to a conventional method on the basis of the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or

- 22 -

8.

The gene fragment may be a part of the non-translated region of the raffinose synthase gene as well as the open reading frame thereof. For example, an oligonucleotide having the same
5 nucleotide sequence as a part of that of 5'-upstream side such as the 1st to 235th nucleotides in the nucleotide sequence of SEQ ID NO: 4, the 1st to 133rd nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like, or a part of that of 3'-downstream side such as the 2588th to 2675th nucleotides in
10 the nucleotide sequence of SEQ ID NO: 4, the 2468th to 2676th nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like.

When PCR is carried out by using the gene fragment as primers, it is possible to amplify a nucleic acid containing
15 raffinose synthase gene from DNA derived from the desired organism (hereinafter referred to as the amplification method of the present invention).

More specifically, for example, oligonucleotides having the nucleotide sequences of the gene fragment are designed
20 and chemically synthesized according to a conventional method.

In general, it is preferred that the number of nucleotides is more from a viewpoint that the specificity of annealing is ensured. It is, however, also preferred that the number of nucleotides is not so many from viewpoints that the primers
25 themselves are liable to have a higher structure giving possible

- 23 -

deterioration of the annealing efficiency and that complicated procedures are required in the purification after the synthesis.

Normally, oligonucleotides composed of 15 to 50 bases are preferred. In this respect, based on the codon table showing the correspondence of amino acids encoded by codons, a mixture of primers can also be synthesized by using a mixture of plural bases so that a residue at a specified position in a primer is changed to different bases according to the variation of codons which can encode one certain amino acid. Alternatively, for example, a base such as inosine which can form a base pair with plural bases can be used instead of the above mixture of plural bases.

Coding Table

Phe: UUU, UUC	Ser: UCU, UCC, UCA, UCG, AGU, AGC
Tyr: UAU, UAC	Cys: UGU, UGC
Stop: UAA, UAG, UGA	Trp: UGG
Leu: UUA, UUG, CUU, CUC, CUA, CUG	Pro: CCU, CCC, CCA, CCG
His: CAU, CAC	Gln: CAA, CAG
Arg: CGU, CGC, CGA, CGG, AGA, AGG	
Ile: AUU, AUC, AUA	Thr: ACU, ACC, ACA, ACG
Asn: AAU, AAC	Lys: AAA, AAG
Met: AUG	
Val: GUU, GUC, GUA, GUG	Ala: GCU, GCC, GCA, GCG
Asp: GAU, GAC	Gly: GGU, GGC, GGA, GGG
Glu: GAA, GAG	

- 24 -

In the above codon table, each codon is shown as the nucleotide sequence in mRNA and its right hand is the 5'-terminus.

U represents uracil base in RNA and corresponds to thymine base in DNA.

5 An oligonucleotide having the same nucleotide sequence as the coding strand of the double-stranded DNA of the gene of the present invention is called a "sense primer" and that having a nucleotide sequence complementary to the coding strand is called an "antisense primer".

10 A sense primer having the same nucleotide sequence as that of 5'-upstream side in the coding strand of the gene of the present invention, and an antisense primer having a nucleotide sequence complementary to the nucleotide sequence on the 3'-downstream side in this coding strand are used in
15 combination for PCR reaction, for example, with a gene library, genomic DNA or cDNA as the template to amplify DNA. As the gene library to be used, for example, there are a cDNA library and a genomic library derived from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed,
20 etc. The gene library may also be a library constructed according to a conventional library construction method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; "Current Protocols In Molecular Biology" (1987), John Wiley &
25 Sons, Inc. ISBN 0-471-50338-X, or a commercially available gene

- 25 -

library as such. As the genomic DNA or cDNA, for example, there are those prepared from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed, etc.

For example, PCR is carried out by using the primers 31 and
5 32 in the above List 3 and as the template cDNA derived from mustard to amplify DNA having the nucleotide sequence represented by the 749th to 1215th nucleotides in the nucleotide sequence of SEQ ID NO: 6. Further, PCR is carried out by using the primers and as the template cDNA derived from rapeseed to
10 amplify DNA having the nucleotide sequence represented by the 1st to 467th nucleotides in the nucleotide sequence of SEQ ID NO: 8. The nucleic acid thus amplified can be confirmed by conventional electrophoresis. The nucleic acid can be cloned according a conventional method such as that described in
15 "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. For the nucleic acid, its restriction map is prepared or its nucleotide sequence is determined by a
20 conventional method, so that the nucleic acid containing raffinose synthase gene or a part thereof can be identified.

When the nucleic acid contains a part of raffinose synthase, PCR can be carried out on the basis of its nucleotide sequence to amplify the nucleic acid containing the 5'-upstream side
25 nucleotide sequence or the 3'-downstream side nucleotide

- 26 -

sequence. That is, based on the nucleotide sequence of the above-obtained nucleic acid, an antisense primer is designed and synthesized for amplification of the 5'-upstream side part, and a sense primer is designed and synthesized for amplification of the 3'-downstream side part. The nucleotide sequence of the 5'-upstream side part or 3'-downstream side part of the nucleotide sequence already obtained can be determined by the RACE method using these primers and a commercially available kit such as Marathon Kit of Clontech. The full length raffinose synthase gene can be obtained by synthesizing new primers based on both terminal sequences in the nucleotide sequence thus determined and carrying out PCR again.

The above detection method of the present invention can also be used in the analysis of genotypes of a plant such as soybean, a Chenopdiaceae plant or a Cruciferae plant, etc.

More specifically, for example, a genomic DNA derived from soybean, a Chenopdiaceae plant or a Cruciferae plant is prepared according to a conventional method, for example, described in "Cloning and Sequence (Plant Biotechnology Experiment Manual)" compiled under the supervision of Itaru Watanabe, edited by Masahiro Sugiura, published by Noson Bunka-sha, Tokyo (1989).

The genomic DNA is digested with at least several kinds of restriction enzymes, followed by electrophoresis. The electrophoresed DNA is blotted on a filter according to a conventional method. This filter is subjected to hybridization

- 27 -

with a probe prepared from DNA having the gene fragment by a conventional method, and DNA to which the probe hybridizes is detected. The DNAs detected are compared in length between different varieties of a specified plant species. The
5 differences in length make possible the analysis of differences in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides between these varieties. Furthermore, when the DNAs detected by the above method are compared in length between the gene recombinant plant and the
10 non-gene recombinant plant of the same variety, the former plant can be distinguished from the latter plant by the detection of hybridizing bands greater in number or higher in concentration for the former plant than for the latter plant. This method can be carried out according to the RFLP (restriction fragment length
15 polymorphism) method, for example, described in "Plant PCR Experiment Protocols" compiled under the supervision of Ko Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 90-94.

Further, the amplification method of the present
20 invention can be used for an analysis of genes of soybean, a Chenopdiaceae plant or a Cruciferae plant, etc. More specifically, for example, the amplification method of the present invention is carried out by using plant genomic DNA prepared from soybean, a Chenopdiaceae plant or a Cruciferae
25 plant to amplify DNA. The amplified DNA is mixed with a

- 28 -

formaldehyde solution, followed by heat denaturing at 85°C for 5 minutes and then quickly cooling on ice. A sample thereof is subjected to electrophoresis on, for example, 6 (w/v)% polyacrylamide gel containing 0 (v/v)% or 10 (v/v)% of glycerol.

5 For this electrophoresis, a commercially available electrophoresis apparatus such as that for SSCP (Single Strand Conformation Polymorphism) can be used and the electrophoresis can be carried out with maintaining the gel at a constant temperature, for example, at 5°C, 25°C, 37°C, etc. From the
10 electrophoresed gel, DNA is detected, for example, by a method such as silver staining method with a commercially available reagent. From the differences of behavior between the varieties in the electrophoresis of the DNA detected, a mutation in the raffinose synthase gene is detected, and an analysis is carried
15 out for differences caused by the mutation in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides. This method can be carried out according to the SSCP method, for example, described in "Plant PCR Experiment Protocols" compiled under the supervision of Ko
20 Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 141-146.

The analysis of the plant gene from soybean, a Chenopdiaceae plant or a Cruciferae plant by the above detection method or amplification method of the present invention can be
25 used not only for the analysis of differences in phenotypic

- 29 -

characteristics accompanied with the expression of raffinose family oligosaccharides, but also, for example, for the selection of clones having the desired characters upon production of a novel variety of soybean, a Chenopdiaceae plant or a Cruciferae plant. Further, it can also be used for identification of a clone thus produced and having the characters derived from a recombinant plant upon producing a plant variety using the recombinant plant.

For expression of the gene of the present invention in cells of a host, preferably, a nucleic acid comprising a nucleic acid fragment which contains the gene of the present invention, and a nucleic acid fragment which has a promoter activity in the host cells and joined to the former nucleic acid fragment (hereinafter referred to as the expression nucleic acid of the present invention) can be used.

The nucleic acid fragment having promoter activity in the expression nucleic acid of the present invention is not limited to a specific one, so long as it is functionable in a host to be transformed. For example, there are synthetic promoters functionable in *Escherichia coli*, such as *E. coli* lactose operon promoter, *E. coli* tryptophan operon promoter and tac promoter, etc.; yeast alcohol dehydrogenase gene (ADH) promoter, adenovirus major late (Ad.ML) promoter, SV40 early promoter, baculovirus promoter and the like. When the host is

- 30 -

a plant, the promoter may include, for example, T-DNA derived constitutive promoters such as nopaline synthase gene (NOS) promoter, octopine synthase gene (OCS) promoter, etc.; plant virus-derived promoters such as cauliflower mosaic virus (CaMV)-derived 19S and 35S promoters; inducible promoters such as phenylalanine ammonia-lyase (PAL) gene promoter, chalcone synthase (CHS) gene promoter, pathogenesis-related protein (PR) gene promoter, etc. Furthermore, vector pSUM-GY1 (see JP-A 06-189777/1994) can also be used, which has a promoter giving specific expression in a specified plant tissue, e.g., a promoter of soybean-derived seed storage protein glycinin gene (JP-A 6-189777).

Furthermore, a nucleic acid fragment having a terminator activity can be joined to the expression nucleic acid of the present invention. In this case, it is generally preferred that the expression nucleic acid of the present invention is constructed so that the nucleic acid fragment having a terminator activity is positioned downstream the raffinose synthase gene. The terminator to be used is not particularly limited, so long as it is functionable in cells of a host to be transformed. For example, when the host is a plant, there are T-DNA derived constitutive terminators such as nopaline synthase gene (NOS) terminator, etc.; plant derived terminators such as terminators of allium virus GV1 or GV2, and the like.

The expression nucleic acid of the present invention

- 31 -

can be introduced into a host cell according to a conventional gene engineering technique to obtain a transformant. If necessary, the expression nucleic acid of the present invention can be inserted into a vector having a suitable marker depending upon a particular transformation technique for introduction of the nucleic acid into a host cell.

A vector into which the expression nucleic acid of the present invention is inserted can be introduced into a microorganism according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. The microorganism transformed with the vector can be selected on the basis of a selection marker such as antibiotic resistance, auxotrophy or the like. In case that the gene of the present invention is joined to the downstream of an inducible promoter (e.g., tac promoter) in the translatable form in the selected microorganism (e.g., *E. coli* transformant), a translated product of the gene of the present invention can be expressed under conventional culture and inducible conditions and can be recovered as a peptide or a protein.

The raffinose synthase activity of the translated product of the gene of the present invention thus prepared can be measured by, for example, a method described in L. Lehle and W. Tanner, Eur. J. Biochem., 38, 103-110 (1973) to identify the

- 32 -

translated product having the "capability of binding D-galactosyl group through α (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in sucrose molecule". More specifically, for example,

5 the gene of the present invention is cloned in pGEX-4T3 (Pharmacia) to obtain a plasmid containing the expression nucleic acid of the present invention. The resultant plasmid is introduced into, for example, *E. coli* HB101 strain to obtain a transformant. The resultant transformant is culture

10 overnight and 1 ml of the culture is inoculated into 100 ml of LB culture medium. It is incubated at 37°C for about 3 hours and IPTG (isopropylthio- β -D-galactoside) is added at a final concentration of 1 mM, followed by further incubation for 5 hours.

Cells are recovered from the culture broth by centrifugation

15 and are suspended by addition of 10 times of the cell weight of 100 mM Tris-HCl (pH 7.4), 1 mM EDTA, 5 mM DTT (dithiothreitol), 1 mM PMSF (phenylmethylsulfonyl fluoride) and 1 mM benzamide.

The suspension is sonicated with a ultrasonic disrupter (Branson) to disrupt the cells. The disrupted cell suspension

20 is centrifuged to recover a soluble protein solution. The resultant protein solution is added to a reaction mixture containing at final concentrations of 100 mM Tris-HCl (pH 7.4), 5 mM DTT (dithiothreitol), 0.01% BSA, 200 μ M sucrose, 5 mM galactinol and 31.7 μ M [^{14} C] sucrose. The reaction mixture is

25 incubated at 37°C, followed by addition of 1.5 times in volume

- 33 -

of ethanol and stirring. Insoluble materials are remove by centrifugation, the supernatant is spotted on, for example, a HPTLC cellulose thin layer chromatography plate (Merch, HPTLC plates cellulose) and then the plate is developed with n-
5 butanol-pyridine-water-acetic acid (60 : 40 : 30 : 3). The developed plate is dried and analyzed with an imaging analyzer (FUJIX Bio-Image Analyzer BAS-2000II manufactured by Fuji Film™) to determine [¹⁴C] raffinose produced to measure the raffinose synthase activity.

10 In addition, the translated product as prepared above can also be used as an antigen for producing an antibody. The antibody thus produced can be used for, for example, detection and determination of the gene of the present invention in a crude protein extract prepared from an organism such as a plant.

15 When the host is a plant, the vector into which the gene of the present invention is inserted can be introduced into plant cells by a conventional means such as *Agrobacterium* infection method (JP-B 2-58917 and JP-A 60-70080),
electroporation into protoplasts (JP-A 60-251887 and JP-B
20 5-68575) or particle gun method (JP-A 5-508316 and JP-A 63-258525). The plant cell transformed by the introduction of the vector can be selected on the basis of a selection marker, for example, resistance to an antibiotic such as kanamycin or hygromycin. From the plant cell thus transformed, a transformed
25 plant can be regenerated by a conventional plant cell cultivation

- 34 -

method, for example, described in "Plant Gene Manipulation Manual (How to Produce Transgenic Plants)" written by Uchimiya, 1990, Kodan-sha Scientific (ISBN 4-06-153513-7), pp. 27-55.

Furthermore, the collection of seeds from the transformed plant
5 also makes it possible to proliferate the transformed plant. In addition, crossing between the transformed plant obtained and the non-transformed plant makes it possible to produce progenic plants with the characters of the transformed plant.

As gene engineering techniques in soybean, basically,
10 the above general techniques can be employed. More specifically, "transformation of soybean plant strain by particle gun" described in EP 301749, gene introduction methods. for example, described in Torisky, R.S., Kovacs, L., Avdiushko, S., Newman, J.D., Hunt, A.G. and Collins, G.B., "Development of a binary
15 vector system for plant transformation based on the supervirulent *Agrobacterium tumefaciens* strain Chry5", Plant Cell Rep., (1997), 17, p. 102-108, etc. can be employed.

As gene engineering techniques in a *Chenopodiaceae* plant, basically, the above general techniques can be employed.
20 More specifically, gene introduction methods, for example, described in M. Mannerlof, S. Tuveesson, P. Steen and P. Tenning, "Transgenic sugar beet tolerant to glyphosate", *Euphytica* (1997), 94, p 83-91, B.K. Konwar, "Agrobacterium tumefaciens-Mediated Genetic Transformation of Sugar Beet
25 (*Beta vulgaris* L.)", J. Plant Biochemistry & Biotechnology

- 35 -

(1994), 3, p. 37-41 can be employed.

As gene engineering techniques in a Cruciferae plant, basically, the above general techniques can be employed. More specifically, the gene introduction can be carried out according to a method, for example, described in J. Fry, A. Barnason and R.B. Horsch, "Transformation of Brassica napus with *Agrobacterium tumefaciens* based vectors", Plant Cell Reports (1987), 6, 321-325.

For example, when gene introduction is carried out by *Agrobacterium* infection method, first, the above-described expression nucleic acid of the present invention is inserted into a binary vector. The resultant vector can be introduced into, for example, *Agrobacterium tumefaciens* LBA 4404 strain which has been converted into a competent state by treatment with calcium chloride. A transformant can be selected by an appropriate selection method according to the selection marker gene of the vector, for example, cultivation of a strain containing the vector in a culture medium containing an antibiotic in case that the selection marker gene is that giving resistance to the antibiotic such as kanamycin. The resultant transformed *Agrobacterium* strain can be culture in a liquid culture medium, for example, LB medium.

Soybean, a Chenopdiaceae plant or a Cruciferae plant can be transformed by using thus obtained *Agrobacterium*

- 36 -

transformant culture broth as described below. For example, seeds from soybean, beet, rapeseed or mustard is sowed aseptically in, for example, 1/2 MS medium containing 2% sucrose and 0.7% agar. After about 1 week, cotyledons and petioles of the germinated plant are cut off with a scalpel aseptically and transplanted in, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D and 3.3 μM AgNO_3 and cultured for one day. The cotyledons and petioles thus precultured are transferred to 1000-fold dilution of the above *Agrobacterium* culture broth and allowed to stand for 5 minutes. The cotyledons and petioles are transferred to the same medium as that of the preculture again and cultured for about 3 to 4 days. The cotyledones and petioles thus cultured are transferred to, for example, MS medium containing 3% sucrose, 4.5 μM BA, 0.05 μM 2,4-D, 3.3 μM AgNO_3 and 500 mg/liter cefotaxim, followed by shaking for 1 day to remove microbial cells. The resultant cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D, 3.3 μM AgNO_3 100 mg/liter cefotaxim and 20 mg/liter kanamycin, followed by culturing for 3 to 4 weeks. Then, the cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5 μM BA, 0.05 μM 2,4-D, 100 mg/liter cefotaxim and 20 mg/liter kanamycin and cultured. Culture in this medium is continued with subculturing every 3 to 4 weeks. When a shoot are regenerated, it is subcultured in,

- 37 -

for example, MS medium containing 3% sucrose, 0.7% agar and 20 mg/liter kanamycin for 3 to 4 weeks. When the plant makes roots, it is transferred to vermiculite-peat moss (1 : 1) and acclimatized by culturing at 21 to 22°C under day and night conditions of 12 hours: 12 hours = day time : night. As the plant grows, it is transferred to appropriate cultivation soil to culture the plant. A genomic DNA is extracted from the leaf of the regenerated plant according to the above method and PCR is carried out by using as primers having partial nucleotide sequences of the expression nucleic acid of the present invention to confirm the insertion of the gene of the present invention into the plant.

As described hereinabove, by introducing the gene of the present invention into a plant, for example, soybean, a Chenopdiaceae plant or a Cruciferae plant, it is possible to vary the expression level and activity of raffinose synthase in the plant to control the amount of raffinose family oligosaccharides in the plant. The gene of the present invention is useful in techniques for varying the expression level and activity of raffinose synthase in soybean, a Chenopdiaceae plant or a Cruciferae plant on the basis of gene homology, for example, techniques such as homologous recombination and antisense technique, cosuppression and the like.

The following examples further illustrate the present

- 38 -

invention in detail but are not to be construed to limit the scope of the present invention.

Example 1

Preparation of cDNA Derived from Soybean

5 About 2 g of immature seeds of soybean (*Glycine max*) Williams82 were frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of
10 chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed
15 with 10 ml of 70% ethanol and then dissolved in 1 ml of elution buffer (10 mM Tris-HCl/pH 7.5, 1 mM EDTA, 0.1% SDS). The solution was allowed to stand at 60°C for 10 minutes and then centrifuged at 10,000 x g for 1 minute to remove insoluble matter. To the resulting supernatant was added an equivalent volume of
20 Oligotex-dT30 (Takara), and the mixture was stirred and then allowed to stand at 65°C for 5 minutes. Further, the mixture was placed on ice and allowed to stand for 3 minutes, to which 200 µl of 5 M NaCl was added, and the mixture was mixed and then allowed to stand at 37°C for 10 minutes. The mixture was then centrifuged

- 39 -

at 10,000 x g for 3 minutes at 4°C. The precipitate was collected and then suspended in 1 ml of TE buffer, and the suspension was allowed to stand at 65°C for 5 minutes. Further, the suspension was placed on ice and then allowing to stand for 3 minutes, followed
5 by centrifugation at 10,000 x g for 3 minutes at 4°C to remove precipitate.

To the resulting supernatant were added 100 µl of 3M sodium acetate and 2 ml of ethanol to precipitate and collect RNA.

The collected RNA was washed twice with 70% ethanol and then
10 dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, First Strand Synthesis Kit for RT-PCR (Amersham) and cDNA Synthesis Kit (Takara) were used, and
15 all operations were made according to the protocol attached to kits.

Example 2

Cloning of Raffinose Synthase Gene from Soybean cDNA

PCR was carried out by using the cDNA obtained from
20 immature seeds of soybean (*Glycine max*) Williams82 in Example 1 as a template and the primers designed on the basis of the amino acid sequence of SEQ ID No: 1, i.e., primers having nucleotide sequences shown in List 4 below to amplify a DNA fragment. The PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal

- 40 -

Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The reaction was carried out by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times to amplify the DNA fragment.

5 The amplified DNA fragment was cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of the nucleotide sequence with a 373S DNA sequencer of ABI. Based on this sequence, primers having nucleotide

10 sequences shown in List 5 below were synthesized. The synthesis of cDNA was carried out with Marathon Kit of Clontech using mRNA obtained from leaves of soybean Williams82 in Example 1. The cDNA obtained was ligated to an adapter contained in the kit with ligase.

These operations were carried out according to the protocol

15 attached to the kit. By using the adapter-ligated cDNA thus prepared, PCR was carried out with the primers shown in List 5 according to the same manner as the above. The nucleotide sequence in terminal region of the gene was analyzed according to the protocol attached to the Marathon Kit of Clontech. As a result,

20 the nucleotide sequence of SEQ ID NO: 2 was determined.

List 4

4-5-F primer:

cgatggatgg giaaittiat icaiccigai tgggaiatgt t 41mer

4-6-RV primer:

- 41 -

ggccacatit tiacia(ag)icc iatiggigci aa 32mer

List 5

5-SC-2:

tggtactagg cgaaacaaga gtagctctga 30mer

5 Example 3

Preparation of cDNA derived from Chenopdiaceae Plant

About 2 g of leaves of beet (*Beta vulgaris*: haming) was frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C.

The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed with 10 ml of 70% ethanol and then dissolved in 180 µl of DEPC-treated sterilized water. The solution was allowed to stand at 55°C for 5 minutes and 20 µl of 5 M NaCl was added thereto. The resulting solution was purified using BIOMAG mRNA PURIFICATION KIT (PerSeptive Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium acetate and ethanol, and RNA was precipitated and collected. The collected RNA was washed twice with 70% ethanol and then dissolved

- 42 -

in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were made according to the protocol attached to the kit.

Example 4

Analysis of Nucleotide Sequence of Raffinose Synthase Gene from Chenopdiaceae Plant

Synthetic DNA primers having the nucleotide sequences shown in List 6 below were synthesized. The PCR method was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cyclor Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech.

The PCR was carried out with the above primers and cDNA of beet obtained in the above Example 3 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. As a result, the combinations of primers 6-3-F and 6-8-RV and primers 6-10-F and 6-6-RV gave an amplification of about 0.3 kb and 0.6 kb bands, respectively. The amplified DNA fragments were cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of nucleotide sequence with a 373S DNA sequencer of ABI. Based on the resulting nucleotide sequences, synthetic DNA

- 43 -

primers having nucleotide sequences shown in List 7 below were prepared and PCR was carried out using cDNA obtained from beet in Example 3 in the same manner as above. As a result, DNA having the nucleotide sequence of SEQ ID NO: 4 was finally obtained from

5 cDNA of beet.

List 6

6-3-F:

cgaggiggit gicciccigg ittigtiati atigaigaig gitggca 47mer

6-8-RV:

10 at(t/c)tt(a/g)tcia cigcia(a/g)(a/g)tc (t/c)tccatigt 29mer

6-10-F:

ggiacitait gg(c/t)ticaigg itgicaiatg gticaigt 38mer

6-6-RV:

ggccacatit tiacia(a/g)icc iatiggigci aa 32mer

15 List 7

7-Sb-1:

atctatattgt catgacgatg atccga 26mer

7-Sb-2RV:

ggccctcatt cccatattgg gatgacctc 30mer

20 7-Sb-3RV:

aagcatgcca aacatacaca tgctcaacag 30mer

7-Sb-4RV:

agaccgagg aaagctttgg gggtactact 30mer

7-Sb-5:

25 tggatgggaa actttataca ccctgact 28mer

- 44 -

7-Sb-6:

gacatgttcc catctacaca cccttgtg 28mer

7-Sb-7:

ccaatttatg ttagtgatgt tgttggcaag 30mer

5 7-Sb-8RV:

tcgactccca gggtagaatt gtcac 26mer

Example 5

Preparation of cDNA Derived from Cruciferae Plant

About 2 g of leaves of mustard (*Brassica juncea*) was frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed with 10 ml of 70% ethanol and then dissolved in 180 µl of DEPC-treated sterilized water. The solution was allowed to stand at 55°C for 5 minutes and to which 20 µl of 5 M NaCl was added. The resulting solution was purified using BIOMAG mRNA PURIFICATION KIT (PerSeptive Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium

- 45 -

acetate and ethanol, and RNA was precipitated and collected. The collected RNA was washed twice with 70% ethanol and then dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were carried out according to the protocol attached to the kit.

In the same manner as described in the above, mRNA was purified from immature seeds of rapeseed Westar (*Brassica napus*) and cDNA was synthesized.

Example 6

Isolation and Nucleotide Sequence Analysis of Raffinose Synthase Gene derived from Cruciferae Plant

DNA primers having the nucleotide sequences shown in List 8 below were synthesized. PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The PCR was carried out with the above primers and cDNA of mustard obtained in Example 5 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. The reaction products were analyzed by agarose gel electrophoresis. As a result, an amplification of about the 1.2 kb bands was detected. The amplified DNA fragment was cloned with TA cloning kit

- 46 -

(Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and nucleotide sequence analysis with a 373S DNA sequencer of ABI.

Based on the resulting nucleotide sequence, synthetic primers
 5 having the nucleotide sequences shown in List 9 below were prepared and PCR was carried out using cDNAs from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*) obtained in Example 5 according to the same manner as the above. As a result, the nucleotide sequence represented by the 749th to 1215th nucleotides
 10 of SEQ ID NO: 6 and by the 1st to 467th nucleotides of SEQ ID NO: 8 were finally determined for cDNA from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*), respectively.

List 8

8-#1:

15 cgattiaaig titggtggac iacicaitgg gtigg 35mer

8-#10RV:

caitgiacca titgicaicc itgia(ag)ccai taigticc 38mer

List 9

9-primer-1:

20 gttagggttc atatgaacac cttcaagctc 30mer

9-primer-2RV:

caacggcgag atcttgcac gtcaac 26mer

Example 7

Nucleotide Sequence Analysis of Raffinose Synthase

- 47 -

Full-Length Gene Derived from Cruciferae Plant

Based on the nucleotide sequences obtained in Example 6, DNA primers having the nucleotide sequences shown in List 10 below were synthesized. The cDNAs from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*) obtained in the same manner as described in Example 5 were ligated to adapters contained in Marathon Kit of Clontech. By using the adapter-ligated cDNAs thus prepared, PCR was carried out with primers shown in List 10. 10-B-2RV, 10-B-3RV and 10-B-4RV primers were used for nucleotide analysis of 5'-termini, and 10-B-1, 10-B-8, 10-B-7 and 10-B-6 primers were used for nucleotide analysis of 3'-termini. The nucleotide sequences were analyzed according to the protocol attached to the Marathon Kit of clontech. As a result, the nucleotide sequence of SEQ ID NO: 6 and SEQ ID NO: 8 were determined from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica napus*), respectively.

List 10

	10-B-2RV:	
	ggattcgaca caaaccgcca cgtcatcgtc	30mer
20	10-B-3RV:	
	ccacgtgcac caccggaact tatcgac	27mer
	10-B-4RV:	
	aacatcgata ccatcggagt catgtccaat	30mer
	10-B-1:	

- 48 -

gtaggggttc atatgaacac cttcaagctc 30mer

10-B-8:

tctacgtctg gcacgcgctt tgcggctac 29mer

10-B-7:

5 gttgacgtca tccacatatt ggagatgttg t 31mer

10-B-6:

gttatcgcta gcatggagca ctgtaatga 29mer

Example 8

Construction of Expression Vectors in Plant for 10 Raffinose Synthase Gene Derived from Cruciferae Plant

Based on the nucleotide sequence of raffinose synthase gene from mustard obtained in Example 7, DNA primers having the nucleotide sequences shown in List 11 were prepared. By using cDNA of mustard, PCR was carried out in the same manner as
15 described in Example 6. The amplified DNA fragment was digested with SacI. The DNA fragment thus digested was ligated to the vector pBI121(-) previously digested with SacI by using Ligation Kit (Takara). Plasmid pBI121 (Clontech) were digested with BamHI and SacI, and ligated to linkers shown in List 12 to prepare
20 the vector pBI121(-). The vector thus obtained was analyzed by a restriction map and PCR using primers having nucleotide sequences shown in List 13, and confirmed the direction of inserted raffinose synthase gene. The vector whose raffinose synthase gene from mustard was inserted in the expressible
25 direction was designated BjRS-Sac(+)-121 and the one whose

- 49 -

raffinose synthase gene from mustard was inserted in the reverse direction was designated BjRS-Sac(-)-121.

List 11

11-SacI-BjN:

5 aacgagctca atccaaaatc tcatcaaata atcgc 35mer

11-SacI-BjintRV:

acaatagttg agggcggaag agtag 25mer

List 12

12-BamSac-(+)linker:

10 gatcgagctc gtgtcggatc cagct 25mer

12-BamSac-(-)linker:

ggatccgaca cgagctc 17mer

List 13

13-35S-3:

15 cctcctcgga ttccattgcc cagctatctg 30mer

13-B-2RV:

ggattcgaca caaaccgcca cgtcatcgtc 30mer

13-B-8:

tctacgtctg gcacgcgctt tgcggctac 29mer

20 Example 9

Transformation with Raffinose Synthase Gene Derived from Cruciferae Plant

The vectors BjRS-Sac(+)-121 and BjRS-Sac(-)-121 prepared in Example 8 were used for the transformation of mustard (Brassica juncea) by the Agrobacterium infection method.

25

- 50 -

Agrobacterium tumefaciens (strain LBA4404 having rifampicin and streptomycin resistance) previously converted into a competent state by calcium chloride treatment was transformed independently with two plasmids BjRS-Sac(+)-121 and
5 BjRS-Sac(-)-121 prepared in Example 8. The transformants were selected on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin by utilizing the kanamycin resistant character conferred by the kanamycin resistant gene (neomycin phosphotransferase, NPTII) of the introduced plasmids.

10 The transformant *Agrobacterium* obtained (*Agrobacterium tumefaciens* strain LBA4404: rifampicin and streptomycin resistant) was cultured on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin at 28°C for a whole day and night, and the culture was used for the transformation
15 of mustard by the method described below.

The seeds of mustard were aseptically sowed on 1/2 MS medium containing 2% sucrose and 0.7% agar. After one week, cotyledons and petioles of sprouting plants were cut out with a scalpel, and transferred to MS medium containing 3% sucrose,
20 0.7% agar, 4.5 µM BA, 0.05 µM 2,4-D and 3.3 µM AgNO₃, followed by preculture for 1 day. The precultured cotyledons and petioles were transferred in a 1000-fold dilution of the *Agrobacterium* culture broth and allowed to stand for 5 minutes. The cotyledons and petioles were transferred again to the same

- 51 -

medium as used in the preculture, and cultured for 3 to 4 days.

The cultured cotyledons and petioles were transferred to MS medium containing 3% sucrose, 4.5 μ M BA, 0.05 μ M 2.4-D, 3.3 μ M AgNO₃ and 500 mg/l cefotaxim, and shaken for 1 day to remove
5 microbial cells. The cotyledons and petioles thus treated were transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5 μ M BA, 0.05 μ M 2.4-D, 3.3 μ M AgNO₃, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultured for 3 to 4 weeks. The cotyledons and petioles were transferred to MS medium containing 3% sucrose,
10 0.7% agar, 4.5 μ M BA, 0.05 μ M 2.4-D, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultivated. The cultivation on this medium was continued with subculturing at intervals of 3 to 4 weeks.

When shoots are began to regenerate, these shoots are subcultured on MS medium containing 3% sucrose, 0.7% agar and
15 20 mg/l kanamycin, and cultivated for 3 to 4 weeks. The rooting plants are transferred to vermiculite : peat moss = 1 : 1, and cultivated at 21°C to 22°C in a cycle of day/night = 12 hours : 12 hours. With the progress of plant body growth, the plants are grown with cultivation soil.

20

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene of the present invention.

- 52 -

SEQ ID NO: 2 shows a nucleotide sequence of the raffinose synthase gene of the present invention.

SEQ ID NO: 3 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from beet.

SEQ ID NO: 4 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from beet.

SEQ ID NO: 5 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from mustard.

SEQ ID NO: 6 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from mustard.

SEQ ID NO: 7 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from rapeseed.

SEQ ID NO: 8 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from rapeseed.

List 1:

The nucleotide sequences shown in List 1 are examples of the typical primers used in the amplification of a DNA fragment having a raffinose synthase gene. All of these sequences are based on the nucleotide sequence of SEQ ID NO: 2. Primer 11 is a sense primer and Primer 12 is an antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

- 53 -

List 2:

The nucleotide sequences shown in List 2 are examples of the typical primers used in the amplification of a cDNA of a raffinose synthase gene. Primer 21 is a sense primer
5 corresponding to the 5'-terminus of the beet-derived raffinose synthase gene. Primer 22 is an antisense primer corresponding to the 3'-terminus. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

10 Primer 23 is a sense primer corresponding to the N-terminus of the open reading frame, and primer 24 is an antisense primer corresponding to the C-terminus.

List 3:

Among the nucleotide sequences shown in List 3, primers
15 31 and 32 are typical primers used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene. Primer 31 is a sense primer used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene from mustard and rapeseed and primer 32 is an
20 antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

Primers 33 and 34 are the typical primers used in the amplification of a cDNA of a raffinose synthase gene of mustard.

25 Primers 33 and 34 are both based on the nucleotide sequence of

- 54 -

raffinose synthase gene in the non-translated region. Primer 33 is a sense primer corresponding to the 5'-terminal non-translated region of the mustard-derived raffinose synthase gene. Primer 34 is an antisense primer corresponding to the 3'-terminal non-translated region.

Primers 35 and 36 are typical primers used in the amplification of an open reading frame coding for the amino acid sequence of a raffinose synthase protein in the cDNA of a raffinose synthase gene. Primer 35 is a sense primer corresponding to the 5'-terminus of the above open reading frame. Primer 36 is an antisense primer corresponding to the 3'-terminus. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

List 4:

The nucleotide sequences shown in List 4 are of the primers used in the cloning of a DNA fragment having the present raffinose synthase gene. The base represented by the symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases is used in the synthesis. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 5:

The nucleotide sequence shown in List 5 is of the primer used in the analysis of a nucleotide sequence of the present

- 55 -

raffinose synthase gene. 5-SC-2 is used in the analysis of the present nucleotide sequence in the 3'-terminal region.

List 6

5 The nucleotide sequences shown in List 6 are of the primers used in the analysis of the present raffinose synthase gene of beet. The base represented by the symbol "i" is inosine.

The bases shown in parentheses mean that a mixture of those bases was used in the synthesis. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an
10 antisense sequence.

List 7

The nucleotide sequences shown in List 7 are of the primers synthesized on the partial nucleotide sequences of the beet raffinose synthase gene. The symbol "RV" as used after the
15 primer number means that the primer referred to by this symbol has an antisense sequence.

List 8:

The nucleotide sequences shown in List 8 are of the primers used in the analysis of the cDNA nucleotide sequence of a raffinose synthase gene of mustard. The base represented by the
20 symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

25

List 9:

- 56 -

The nucleotide sequences shown in List 9 are of the primers synthesized on the partial nucleotide sequences of the mustard raffinose synthase gene. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 10:

The nucleotide sequences shown in List 10 are of the primers used in the analysis of the nucleotide sequences of raffinose synthase gene of mustard and rapeseed. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 11:

The nucleotide sequences shown in List 11 are of the primers used in the amplification of 5'-terminal region of a mustard raffinose synthase gene. 11-SacI-BjN is a primer whose SacI restriction site is added to the nucleotide sequence represented by the 4th to 29th nucleotides in SEQ ID NO: 6. 11-SacI-BjintRV is an antisense primer having a nucleotide sequence corresponding to the nucleotide sequence represented by the 1164th to 1188th nucleotides in SEQ ID NO: 6.

List 12:

The nucleotide sequences shown in List 12 are of the adapters added to a mustard cDNA. These synthetic DNA take a double-stranded form when mixed together because they are complementary strands. This adapter has cohesive ends of cleavage

- 57 -

sites for the restriction enzymes BamHI and SacI on both termini, and contains the restriction sites for the restriction enzymes BamHI and SacI in the double-stranded region.

List 13:

5 The nucleotide sequences shown in List 13 are of the primers used in the confirmation of inserting direction of the mustard-derived raffinose synthase gene. 13-35S-3 is a primer of sense to 35S promoter. 13-B-2RV is an antisense primer having the nucleotide sequence represented by the 593rd to 622nd nucleotides
10 of SEQ ID NO: 6, 13-B-8 is a sense primer having the nucleotide sequence represented by the 1110th to 1138th nucleotides in SEQ ID NO: 6.

As described hereinabove, according to the present
15 invention, it is possible to provide raffinose synthase genes which can be utilized in techniques for varying expression level and activity of raffinose synthase in plants.

SEQUENCE LISTING FREE TEXT

20 SEQ ID NO: 9 to SEQ ID NO: 20: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 21 and SEQ ID NO: 22: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

25 SEQ ID NO: 23: Designated oligonucleotide primer to

- 58 -

obtain raffinose synthase gene.

SEQ ID NO: 24 to SEQ ID NO: 27: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, y is t or c, r is a or g.

5 SEQ ID NO: 28 to SEQ ID NO: 35: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 36 and SEQ ID NO: 37: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

10 SEQ ID NO: 38 to SEQ ID NO: 48: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 49 and SEQ ID NO: 50: Designed oligonucleotide linker to obtain raffinose synthase gene.

15 SEQ ID NO: 51 to SEQ ID NO: 53: Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44MB
(B) COMPUTER: IBM PC
(C) OPERATING SYSTEM: DOS 5.0
(D) SOFTWARE: ASCII

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 10/120550
(B) FILING DATE: 1998-04-30
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 10/120551
(B) FILING DATE: 1998-04-30
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 10/345590
(B) FILING DATE: 1998-12-10
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 10/351246
(B) FILING DATE: 1998-12-10
(C) CLASSIFICATION:

- 60 -

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	Lys	Leu	Lys	Leu	Leu	Lys	Tyr	Thr	Glu	Ser	Val	Glu	Val	Ser	Leu	Glu
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20	Tyr	Val	Asp	Arg	Thr	Val	Arg	Leu	Gln	Val	Ser	Trp	Pro	Cys	Ser	Ser
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				Phe Lys Leu Leu Lys Lys Leu Val Leu Pro Asp Gly Ser Ile Leu Arg															
				35					40					45					
5				tgt caa cat tat gca ctt ccc acc cga gac tgc tta ttt gta gat cct															190
				Cys Gln His Tyr Ala Leu Pro Thr Arg Asp Cys Leu Phe Val Asp Pro															
				50					55					60					
				tta cat gat ggg aaa aca atg ctc aaa att tgg aac ctc aat aaa tgt															238
				Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp Asn Leu Asn Lys Cys															
10				65					70					75					
				tcc ggg gtt ttg ggt ctg ttc aat tgc caa gga gga ggt tgg tgc cct															286
				Ser Gly Val Leu Gly Leu Phe Asn Cys Gln Gly Gly Gly Trp Cys Pro															
				80					85					90					95
				gtt act agg cga aac aag agt agc tct gac tat tca cac tcc gtg act															334
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				100					105										110
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25				145					150					155					
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				Glu Pro Phe Ser Cys Glu Leu Leu Thr Val Ser Pro Val Val Ile Leu															
				160					165					170					175
				ccc aga aaa tca atc caa ttt gcc cca att gga ttg gta aac atg ctc															574
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				Ser Glu Lys Pro Glu Ser Val Lys Ile Asp Gly Glu Ser Val Glu Phe															

- 62 -

	225	230	235	
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	Ser Arg Leu Ser Val Val Glu Tyr Leu Phe			
	260	265		
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	Ser Asn Phe Met Val Asn Gly His Val Ile Leu Ser Gln Val Pro Ser			
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25	Phe Asp Ala Pro Glu Pro Lys Ala Arg His Val Val Ser Val Gly Gln			
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	Ile Val Ile Leu Pro Leu Ile Glu Gly Pro Phe Arg Ala Ser Leu Gln			
		130	135	140
35	Pro Gly Ser Val Asp Asp Tyr Val Asp Ile Cys Val Glu Ser Gly Ser			
		145	150	155
	Thr Lys Val Val Gly Asp Ser Phe Arg Ala Val Leu Tyr Ile Arg Ala			
		165	170	175
	Gly Pro Asp Pro Phe Lys Leu Ile Lys Asp Thr Met Lys Glu Val Gln			

- 63 -

			180					185					190			
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	Gly	Val	Pro	Pro	Gly	Leu	Val	Leu	Ile	Asp	Asp	Gly	Trp	Gln	Ser	Ile
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10	Cys	His	Asp	Asp	Asp	Pro	Ile	Thr	Asp	Gln	Glu	Gly	Ile	Asn	Arg	Thr
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	385					390					395					400
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				420					425					430		
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			435					440					445			
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		450					455					460				
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	Gly	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His
					485					490					495	

- 64 -

	Cys	Ala	Tyr	Asn	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	His	Pro	Asp	Trp
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	Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Glu	Phe	His	Ala	Ala	Ser
			515					520					525			
5	Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Val	Ser	Asp	Val	Val	Gly	Lys
		530					535					540				
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	545					550					555					560
	Leu	Arg	Cys	Glu	Tyr	His	Ala	Leu	Pro	Thr	Lys	Asp	Cys	Leu	Phe	Val
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				580					585					590		
	Lys	Tyr	Asn	Gly	Val	Leu	Gly	Val	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp
			595					600					605			
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		610					615					620				
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	Lys	Pro	Phe	Pro	Ile	Lys	Gly	Val	Glu	Cys	Phe	Ala	Met	Tyr	Phe	Thr
20					645					650					655	
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				660					665					670		
	Ser	Leu	Asp	Pro	Phe	Asp	Tyr	Glu	Leu	Ile	Val	Val	Ser	Pro	Met	Thr
			675					680					685			
25	Ile	Leu	Pro	Trp	Glu	Ser	Ile	Ala	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn
		690					695					700				
	Met	Leu	Asn	Ala	Gly	Gly	Ala	Val	Lys	Ser	Leu	Asp	Ile	Ser	Glu	Asp
	705					710					715					720
	Asn	Glu	Asp	Lys	Met	Val	Gln	Val	Gly	Ile	Lys	Gly	Ala	Gly	Glu	Met
30					725					730					735	
	Met	Val	Tyr	Ser	Ser	Glu	Lys	Pro	Lys	Ala	Cys	Arg	Val	Asn	Gly	Glu
				740					745					750		
	Asp	Met	Glu	Phe	Glu	Tyr	Glu	Glu	Ser	Met	Ile	Lys	Val	Gln	Val	Thr
			755				760					765				
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- 65 -

<212> DNA

<213> Beta vulgaris L.

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	ttgtggggat atttataact atcatattat ttgtgtagat cattctacaa aaaagagagt	180
	gagttttttt agctcttatt tcctaagaaa ttaatagcaa aagttttgca taact atg	238
		Met
	gct cca agc ttt agc aag gaa aat tcc aag acg tgt gat gag gtt gca	286
15	Ala Pro Ser Phe Ser Lys Glu Asn Ser Lys Thr Cys Asp Glu Val Ala	
	5 10 15	
	aac cat gat gat tgc aac acg tgt cca ata att tcc ttg gaa gaa tca	334
	Asn His Asp Asp Cys Asn Thr Cys Pro Ile Ile Ser Leu Glu Glu Ser	
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20	aac ttc atg gtg aat ggt cac gtg ata ttg tcc caa gtt cca tcc aac	382
	Asn Phe Met Val Asn Gly His Val Ile Leu Ser Gln Val Pro Ser Asn	
	35 40 45	
	atc acg gcc att agt aaa atg ggt ttt gat ggg ctt ttt gtg ggt ttt	430
	Ile Thr Ala Ile Ser Lys Met Gly Phe Asp Gly Leu Phe Val Gly Phe	
25	50 55 60 65	
	gat gct cca gag ccc aag gcc cgg cac gtt gta tcc gtg ggc cag ctc	478
	Asp Ala Pro Glu Pro Lys Ala Arg His Val Val Ser Val Gly Gln Leu	
	70 75 80	
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30	Lys Gly Ile Pro Phe Met Ser Ile Phe Arg Phe Lys Val Trp Trp Thr	
	85 90 95	
	acc cat tgg act ggg tcc aat ggg cgg gac ctt gag cat gag acc caa	574
	Thr His Trp Thr Gly Ser Asn Gly Arg Asp Leu Glu His Glu Thr Gln	
	100 105 110	
35	att ctc atc ctt gat aag tca gat gaa ggt ttg ggc cgt ccc tat att	622
	Ile Leu Ile Leu Asp Lys Ser Asp Glu Gly Leu Gly Arg Pro Tyr Ile	
	115 120 125	
	gtg atc ctc cca ttg atc gaa ggc cca ttt cgg gca tct ctc cag ccg	670
	Val Ile Leu Pro Leu Ile Glu Gly Pro Phe Arg Ala Ser Leu Gln Pro	

- 66 -

	130		135		140		145	
	ggt tct gtt gat gac tat gtg gat ata tgt gtt gag agt ggg tcc act							718
	Gly Ser Val Asp Asp Tyr Val Asp Ile Cys Val Glu Ser Gly Ser Thr							
		150		155		160		
5	aaa gtt gtc gga gac tcg ttc cgg gct gtt ctt tat ata cgg gct ggg							766
	Lys Val Val Gly Asp Ser Phe Arg Ala Val Leu Tyr Ile Arg Ala Gly							
		165		170		175		
	cct gac cca ttt aag tta att aaa gat aca atg aag gaa gtc caa gcc							814
	Pro Asp Pro Phe Lys Leu Ile Lys Asp Thr Met Lys Glu Val Gln Ala							
10		180		185		190		
	cat tta ggg act ttc aaa ctc tta gat gac aaa act cct cca gga ata							862
	His Leu Gly Thr Phe Lys Leu Leu Asp Asp Lys Thr Pro Pro Gly Ile							
		195		200		205		
	gtg gac aag ttt gga tgg tgt aca tgg gat gca ttt tac ctc aaa gta							910
15	Val Asp Lys Phe Gly Trp Cys Thr Trp Asp Ala Phe Tyr Leu Lys Val							
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	gag ccw tat ggt gtt tgg gaa gga gtt aaa gga ctc gtc gaa aac ggg							958
	Glu Pro Tyr Gly Val Trp Glu Gly Val Lys Gly Leu Val Glu Asn Gly							
		230		235		240		
20	gtc cca ccc ggt ctc gta ctc att gat gat ggg tgg caa tct att tgt							1006
	Val Pro Pro Gly Leu Val Leu Ile Asp Asp Gly Trp Gln Ser Ile Cys							
		245		250		255		
	cat gac gat gat ccg att acc gac caa gaa ggg ata aac cgg act tct							1054
	His Asp Asp Asp Pro Ile Thr Asp Gln Glu Gly Ile Asn Arg Thr Ser							
25		260		265		270		
	gcc ggc gag caa atg cca tgt aga ttg atc aag tac gag gaa aac ttc							1102
	Ala Gly Glu Gln Met Pro Cys Arg Leu Ile Lys Tyr Glu Glu Asn Phe							
		275		280		285		
	aag ttt agg gac tat aaa agc cca aat att atg ggc cat gag gat cat							1150
30	Lys Phe Arg Asp Tyr Lys Ser Pro Asn Ile Met Gly His Glu Asp His							
		290		295		300		305
	ccc aat atg gga atg agg gcc ttt gtt agg gac ctt aag gag gag ttc							1198
	Pro Asn Met Gly Met Arg Ala Phe Val Arg Asp Leu Lys Glu Glu Phe							
		310		315		320		
35	aaa act gtt gag cat gtg tat gtt tgg cat gct ttt acg ggc tat tgg							1246
	Lys Thr Val Glu His Val Tyr Val Trp His Ala Phe Thr Gly Tyr Trp							
		325		330		335		
	gga ggg gta agg ccc aat gtt cca ggc cta ccr gag gcc caa gta gta							1294
	Gly Gly Val Arg Pro Asn Val Pro Gly Leu Pro Glu Ala Gln Val Val							

- 68 -

					550					555					560		
		cgt	tgc	gag	tac	cat	gca	ctt	cct	act	aag	gat	tgc	cta	ttt	gta	gat
		Arg	Cys	Glu	Tyr	His	Ala	Leu	Pro	Thr	Lys	Asp	Cys	Leu	Phe	Val	Asp
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		Pro	Leu	His	Asp	Gly	Lys	Thr	Met	Leu	Lys	Ile	Trp	Asn	Leu	Asn	Lys
					580					585					590		
		tac	aat	gga	gtg	ctt	gga	gtc	ttc	aat	tgc	caa	gga	gga	ggg	tgg	agc
		Tyr	Asn	Gly	Val	Leu	Gly	Val	Phe	Asn	Cys	Gln	Gly	Gly	Gly	Trp	Ser
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		Arg	Glu	Ser	Arg	Lys	Asn	Leu	Cys	Phe	Ser	Glu	Tyr	Ser	Lys	Pro	Ile
		610					615					620				625	
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15		Ser	Cys	Lys	Thr	Ser	Pro	Lys	Asp	Val	Glu	Trp	Glu	Asn	Gly	His	Lys
					630					635					640		
		cca	ttc	ccc	atc	aaa	gga	gtg	gaa	tgt	ttt	gcc	atg	tac	ttc	acc	aag
		Pro	Phe	Pro	Ile	Lys	Gly	Val	Glu	Cys	Phe	Ala	Met	Tyr	Phe	Thr	Lys
					645					650					655		
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		Glu	Lys	Lys	Leu	Ile	Leu	Ser	Gln	Leu	Ser	Asp	Thr	Ile	Glu	Ile	Ser
					660					665					670		
		ctt	gat	ccc	ttc	gat	tac	gag	ctt	att	gta	gtc	tct	ccg	atg	aca	att
		Leu	Asp	Pro	Phe	Asp	Tyr	Glu	Leu	Ile	Val	Val	Ser	Pro	Met	Thr	Ile
25			675						680						685		
		cta	ccc	tgg	gag	tcg	atc	gca	ttt	gca	ccc	ata	gga	tta	gta	aac	atg
		Leu	Pro	Trp	Glu	Ser	Ile	Ala	Phe	Ala	Pro	Ile	Gly	Leu	Val	Asn	Met
		690					695					700				705	
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30		Leu	Asn	Ala	Gly	Gly	Ala	Val	Lys	Ser	Leu	Asp	Ile	Ser	Glu	Asp	Asn
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		gag	gat	aag	atg	gtt	cag	gtt	ggt	att	aaa	ggg	gcc	gga	gaa	atg	atg
		Glu	Asp	Lys	Met	Val	Gln	Val	Gly	Ile	Lys	Gly	Ala	Gly	Glu	Met	Met
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		Val	Tyr	Ser	Ser	Glu	Lys	Pro	Lys	Ala	Cys	Arg	Val	Asn	Gly	Glu	Asp
					740					745					750		
		atg	gag	ttt	gag	tat	gaa	gag	agc	atg	att	aag	gtt	caa	gtt	aca	tgg
		Met	Glu	Phe	Glu	Tyr	Glu	Glu	Ser	Met	Ile	Lys	Val	Gln	Val	Thr	Trp

- 69 -

	755		760		765	
	aac cat aac tca ggt ggt ttt acc act gtt gag tac tta ttt tga gcttg					2592
	Asn His Asn Ser Gly Gly Phe Thr Thr Val Glu Tyr Leu Phe					
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		20		25		30
	Ala Asn Gly His Val Val Leu Thr Asp Val Pro Val Asn Val Thr Val					
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20	Thr Ala Ser Pro Tyr Leu Ala Asp Lys Asp Gly Glu Pro Val Asp Ala					
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	Ser Ala Gly Ser Phe Ile Gly Phe Asn Leu Asp Gly Glu Pro Arg Ser					
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	Arg His Val Ala Ser Ile Gly Lys Leu Arg Asp Ile Arg Phe Met Ser					
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	Ile Phe Arg Phe Lys Val Trp Trp Thr Thr His Trp Val Gly Ser Lys					
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	Gly Ser Asp Ile Glu Asn Glu Thr Gln Ile Ile Ile Leu Glu Asn Ser					
		115		120		125
30	Gly Ser Gly Arg Pro Tyr Val Leu Leu Leu Pro Leu Leu Glu Gly Ser					
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	Phe Arg Ser Ser Phe Gln Pro Gly Glu Asp Asp Asp Val Ala Val Cys					
		145		150		155
	Val Glu Ser Gly Ser Thr Gln Val Thr Gly Ser Glu Phe Arg Gln Val					
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	Val Tyr Val His Ala Gly Asp Asp Pro Phe Lys Leu Val Lys Asp Ala					
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	Met Lys Val Val Arg Val His Met Asn Thr Phe Lys Leu Leu Glu Glu					
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- 70 -

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	Ala	Phe	Tyr	Leu	Thr	Val	Asn	Pro	Asp	Gly	Val	His	Lys	Gly	Val	Lys
	225					230				235						240
5	Cys	Leu	Val	Asp	Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu	Ile	Asp	Asp
					245					250					255	
	Gly	Trp	Gln	Ser	Ile	Gly	His	Asp	Ser	Asp	Gly	Ile	Asp	Val	Glu	Gly
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	Met	Ser	Cys	Thr	Val	Ala	Gly	Glu	Gln	Met	Pro	Cys	Arg	Leu	Leu	Lys
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	290						295					300				
	Asn	Glu	Val	Gly	Met	Lys	Ala	Phe	Val	Arg	Asp	Leu	Lys	Glu	Glu	Phe
	305					310					315					320
15	Ser	Thr	Val	Asp	Tyr	Ile	Tyr	Val	Trp	His	Ala	Leu	Cys	Gly	Tyr	Trp
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20			355					360					365			
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25	Ile	Asp	Gly	Val	Lys	Val	Asp	Val	Ile	His	Ile	Leu	Glu	Met	Leu	Cys
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	Glu	Lys	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	Phe	Lys	Ala
				420					425					430		
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30			435					440					445			
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	450						455					460				
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	465					470					475					480
35	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	Met	Val	His	Cys
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5	Asp Phe Asp Leu Leu Lys Arg Leu Val Leu Pro Asp Gly Ser Ile Leu			
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	Arg Cys Glu His Tyr Ala Leu Pro Thr Arg Asp Arg Leu Phe Glu Asp			
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10	Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly Gly Gly Trp Cys			
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	Arg Glu Thr Arg Arg Asn Gln Cys Phe Ser Gln Cys Val Asn Thr Leu			
	610	615	620	
	Thr Ala Thr Thr Asn Pro Lys Asp Val Glu Trp Asn Ser Gly Asn Asn			
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	Pro Ile Ser Val Glu Asn Val Glu Glu Phe Ala Leu Phe Leu Ser Gln			
	645	650	655	
	Ser Lys Lys Leu Val Leu Ser Gly Pro Asn Asp Asp Leu Glu Ile Thr			
	660	665	670	
20	Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser Pro Val Val Thr			
	675	680	685	
	Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly Leu Val Asn Met			
	690	695	700	
	Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr His Glu Glu Ser			
25	705	710	715	720
	Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg Val Tyr Ala Ser			
	725	730	735	
	Arg Lys Pro Ala Ser Cys Lys Ile Asp Gly Glu Val Val Glu Phe Gly			
	740	745	750	
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	755	760	765	
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- 72 -

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 tctatcagcg aaa atg gct cca ccg agc gta att aaa tcc gat gct gca 169
 Met Ala Pro Pro Ser Val Ile Lys Ser Asp Ala Ala

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 gtc aac ggc att gac ctc tcc gga aag ccg ctt ttc cgg cta gag ggt 217
 Val Asn Gly Ile Asp Leu Ser Gly Lys Pro Leu Phe Arg Leu Glu Gly
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 tcc gat ctc cta gcc aat ggt cac gtt gtc tta acc gat gta ccg gtt 265
 Ser Asp Leu Leu Ala Asn Gly His Val Val Leu Thr Asp Val Pro Val
 30 35 40
 aac gtg act gtc act gct tca cct tac cta gct gac aaa gac gga gaa 313
 Asn Val Thr Val Thr Ala Ser Pro Tyr Leu Ala Asp Lys Asp Gly Glu
 45 50 55 60
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 Pro Val Asp Ala Ser Ala Gly Ser Phe Ile Gly Phe Asn Leu Asp Gly
 65 70 75
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 25 cga ttc atg agc ata ttc cgt ttc aag gtt tgg tgg act act cac tgg 457
 Arg Phe Met Ser Ile Phe Arg Phe Lys Val Trp Trp Thr Thr His Trp
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 Val Gly Ser Lys Gly Ser Asp Ile Glu Asn Glu Thr Gln Ile Ile Ile
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 125 130 135 140
 35 ctt gaa ggc tct ttc cgt tca tcc ttt cag cct ggg gaa gac gat gac 601
 Leu Glu Gly Ser Phe Arg Ser Ser Phe Gln Pro Gly Glu Asp Asp Asp
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 Val Ala Val Cys Val Glu Ser Gly Ser Thr Gln Val Thr Gly Ser Glu

- 73 -

		160		165		170		
		ttt cgt caa gtt gtg tat gtt cac gcc gga gac gat ccg ttc aag ctc						697
		Phe Arg Gln Val Val Tyr Val His Ala Gly Asp Asp Pro Phe Lys Leu						
		175		180		185		
5		gtg aaa gac gcg atg aag gtg gtt agg gtt cat atg aac acc ttc aag						745
		Val Lys Asp Ala Met Lys Val Val Arg Val His Met Asn Thr Phe Lys						
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		ctc ttg gaa gag aag acg ccg ccg gga atc gtc gat aag ttc ggg tgg						793
		Leu Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp						
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		cta atc gac gac ggt tgg caa tcg att gga cat gac tcc gat ggt atc						937
		Leu Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile						
		255		260		265		
20		gat gtt gaa ggg atg agt tgt acc gtc gcc ggg gag caa atg cct tgc						985
		Asp Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys						
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		agg ctt ctg aaa ttt caa gag aac ttc aag ttc aga gac tac gtc tct						1033
		Arg Leu Leu Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser						
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		Pro Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu						
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30		Lys Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu						
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		Cys Gly Tyr Trp Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro						
		335		340		345		
35		tca act att gtc cgg cca gag ctc tcg ccg ggg ctt aag ttg acg atg						1225
		Ser Thr Ile Val Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met						
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		caa gat ctc gcc gtt gat aag att gtc gat acc gga atc gga ttc gtc						1273
		Gln Asp Leu Ala Val Asp Lys Ile Val Asp Thr Gly Ile Gly Phe Val						

- 74 -

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	Glu	Met	Leu	Cys	Glu	Lys	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	
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	Tyr	Phe	Lys	Ala	Leu	Thr	Ser	Ser	Val	Asn	Lys	His	Phe	Asp	Gly	Asn	
		430					435					440					
	ggc	gtt	atc	gct	agc	atg	gag	cac	tgt	aat	gat	ttc	atg	ttc	ctt	gga	1513
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	445					450					455				460		
	acc	gaa	gcc	atc	tct	cta	ggt	cgt	gtc	ggt	gat	gac	ttt	tgg	tgc	acg	1561
	Thr	Glu	Ala	Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	
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	Asp	Pro	Ser	Gly	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	
			480					485					490				
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	Met	Val	His	Cys	Ala	Tyr	Asn	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	Gln	
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	Pro	Asp	Trp	Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Glu	Phe	His	
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	Gly	Ser	Ile	Leu	Arg	Cys	Glu	His	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Arg	
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- 75 -

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	Asn Leu Asn Lys Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly			
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5	gga gga tgg tgc aga gaa acc cga cgc aac caa tgc ttc tcc caa tgc			1993
	Gly Gly Trp Cys Arg Glu Thr Arg Arg Asn Gln Cys Phe Ser Gln Cys			
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	gtt aac acg tta acc gcc aca aca aat cct aag gac gtt gaa tgg aac			2041
	Val Asn Thr Leu Thr Ala Thr Thr Asn Pro Lys Asp Val Glu Trp Asn			
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	agt ggg aac aac cca atc tcc gtt gaa aac gtt gaa gag ttt gct ttg			2089
	Ser Gly Asn Asn Pro Ile Ser Val Glu Asn Val Glu Glu Phe Ala Leu			
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	Phe Leu Ser Gln Ser Lys Lys Leu Val Leu Ser Gly Pro Asn Asp Asp			
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	ctc gag atc act ttg gag cct ttc aag ttt gag cta atc act gtc tca			2185
	Leu Glu Ile Thr Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser			
	670	675	680	
20	cca gtt gtc act att gag ggt agt tcg gtt cag ttt gct cca atc gga			2233
	Pro Val Val Thr Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly			
	685	690	695	700
	ttg gtt aac atg cta aac act agc ggt gca att cga tcc ttg gtg tat			2281
	Leu Val Asn Met Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr			
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	cat gag gaa tcc gtt gag att gga gtt cgt ggt gct gga gag ttc agg			2329
	His Glu Glu Ser Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg			
	720	725	730	
30	gtt tat gca tca agg aaa cct gcg agc tgc aaa att gat ggt gaa gtt			2377
	Val Tyr Ala Ser Arg Lys Pro Ala Ser Cys Lys Ile Asp Gly Glu Val			
	735	740	745	
	gtt gag ttt gga tac gaa gag tca atg gtg atg gtt caa gtg cct tgg			2425
	Val Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp			
	750	755	760	
35	tct gca ccc gag ggt ttg tct tct att aag tat gag ttt tag agtttccga			2476
	Ser Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Glu Phe			
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	aggtgcttat ttgtatcctt ctaaactcct taattatgag ctccgtgccg tttctttttc			2536
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	Gly	Val	Lys	Cys	Leu	Val	Asp	Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu
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	Ile	Asp	Asp	Gly	Trp	Gln	Ser	Ile	Gly	His	Asp	Ser	Asp	Gly	Ile	Asp
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	Val	Glu	Gly	Met	Ser	Cys	Thr	Val	Ala	Gly	Glu	Gln	Met	Pro	Cys	Arg
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20	Leu	Pro	Lys	Phe	Gln	Glu	Asn	Phe	Lys	Phe	Arg	Asp	Tyr	Val	Ser	Pro
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	Lys	Asp	Lys	Asn	Glu	Val	Gly	Met	Lys	Ala	Phe	Val	Arg	Asp	Leu	Lys
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	Glu	Glu	Phe	Ser	Thr	Val	Asp	Tyr	Ile	Tyr	Val	Trp	His	Ala	Leu	Cys
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				165					170						175	
	Pro	Asp	Met	Ala	Asn	Glu	Phe	Tyr	Glu	Gly	Leu	His	Ser	His	Leu	Gln
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35			195					200					205			
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	225					230					235					240

- 77 -

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	Glu	Ala	Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	
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	Leu	Ser	Gln	Ser	Lys	Lys	Leu	Val	Leu	Ser	Gly	Gln	Asn	Asp	Asp	Leu	
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	Glu	Ile	Thr	Leu	Glu	Pro	Phe	Lys	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	
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	Val	Val	Thr	Ile	Glu	Gly	Ser	Ser	Val	Gln	Phe	Ala	Pro	Ile	Gly	Leu	
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	Val	Asn	Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Arg	Ser	Leu	Val	Tyr	His	
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	Tyr	Ala	Ser	Lys	Lys	Pro	Val	Ser	Cys	Lys	Ile	Asp	Gly	Glu	Asp	Val	
		530				535				540							
	Glu	Phe	Gly	Tyr	Glu	Glu	Ser	Met	Val	Met	Val	Gln	Val	Pro	Trp	Ser	

- 78 -

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	Thr	Trp	Asp	Ala	Phe	Tyr	Leu	Thr	Val	Asn	Pro	Asp	Gly	Val	His	Lys	
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	Gly	Val	Lys	Cys	Leu	Val	Asp	Gly	Gly	Cys	Pro	Pro	Gly	Leu	Val	Leu	
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		50					55					60					
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	Leu	Pro	Lys	Phe	Gln	Glu	Asn	Phe	Lys	Phe	Arg	Asp	Tyr	Val	Ser	Pro	
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	aaa	gac	aaa	aac	gaa	gtc	ggg	atg	aaa	gct	ttc	gtc	aga	gat	ctg	aaa	336
	Lys	Asp	Lys	Asn	Glu	Val	Gly	Met	Lys	Ala	Phe	Val	Arg	Asp	Leu	Lys	
35				100					105					110			
	gaa	gaa	ttc	tcc	acc	gtt	gat	tac	atc	tac	gtc	tgg	cac	gcg	ctt	tgc	384
	Glu	Glu	Phe	Ser	Thr	Val	Asp	Tyr	Ile	Tyr	Val	Trp	His	Ala	Leu	Cys	
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	ggy	tac	tgg	ggw	ggt	ctt	cgt	ccc	gga	gct	cct	act	ctt	ccg	ccs	tcg	432

- 79 -

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	gat	ctc	gcc	gtt	gat	aag	atc	atc	gat	acc	gga	atc	gga	ttc	gtc	tcg	528
	Asp	Leu	Ala	Val	Asp	Lys	Ile	Ile	Asp	Thr	Gly	Ile	Gly	Phe	Val	Ser	
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10	Pro	Asp	Met	Ala	Asn	Glu	Phe	Tyr	Glu	Gly	Leu	His	Ser	His	Leu	Gln	
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	Asn	Val	Gly	Ile	Asn	Gly	Val	Lys	Val	Asp	Val	Ile	His	Ile	Leu	Glu	
			195					200					205				
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	Met	Leu	Cys	Glu	Lys	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	
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	ttc	aag	gcg	tta	acg	tcg	tca	gtg	aat	aag	cat	ttt	gac	ggc	aac	gcc	720
	Phe	Lys	Ala	Leu	Thr	Ser	Ser	Val	Asn	Lys	His	Phe	Asp	Gly	Asn	Ala	
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	Val	Ile	Ala	Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	
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	Pro	Ser	Gly	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	Met	
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30	gtc	cac	tgt	gcc	tac	aac	agt	ctt	tgg	atg	gga	aat	ttc	atc	cag	cct	912
	Val	His	Cys	Ala	Tyr	Asn	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	Gln	Pro	
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	Asp	Trp	Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Glu	Phe	His	Ala	
35	305					310					315				320		
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	Ala	Ser	Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Ile	Ser	Asp	Cys	Val	
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	ggc	cag	cac	gat	ttc	gat	ctc	ttg	agg	aga	ctc	gtt	ttg	cct	gac	ggg	1056

- 80 -

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	Val	Asn	Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Arg	Ser	Leu	Val	Tyr	His	
				500				505					510				
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	Tyr	Ala	Ser	Lys	Lys	Pro	Val	Ser	Cys	Lys	Ile	Asp	Gly	Glu	Asp	Val	
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	ggaacaaagt tatgcactat tatttaaggt	30
30	<210> 11	
	<211> 27	
	<212> DNA	
	<213> Artificial Sequence	
35	<220>	
	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
	<400> 11	
	ctaccaaatt ccacaactta aagttca	27

- 82 -

	<210> 12	
	<211> 32	
	<212> DNA	
5	<213> Artificial Sequence	
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	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
10	<400> 12	
	ggaataataa gcttcacaca tactgtactc tc	32
	<210> 13	
	<211> 30	
15	<212> DNA	
	<213> Artificial Sequence	
	<220>	
	<223> Designed oligonucleotide primer to obatin raffinose synthase gene.	
20	<400> 13	
	atggctccaa gctttagcaa ggaaaattcc	30
	<210> 14	
25	<211> 30	
	<212> DNA	
	<213> Artificial Sequence	
	<220>	
30	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
	<400> 14	
	tcaaaataag tactcaacag tggtaaaacc	30
35	<210> 15	
	<211> 30	
	<212> DNA	
	<213> Artificial Sequence	

- 83 -

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 15

5 ttggaagaga agacgccgcc gggaatcgtc 30

<210> 16

<211> 30

<212> DNA

10 <213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

15 <400> 16

ttaagccccg gcgagagctc tggccggaca 30

<210> 17

<211> 30

20 <212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

25

<400> 17

accaatccaa aatctcatca aataatcgca 30

<210> 18

30 <211> 25

<212> DNA

<213> Artificial Sequence

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35 <223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 18

aaataatagg ggcagtacaa attacaccac 30

	<210> 19	
	<211> 29	
	<212> DNA	
	<213> Artificial Sequence	
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	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
	<400> 19	
10	atggctccac cgagcgtaat taaatccga	29
	<210> 20	
	<211> 30	
	<212> DNA	
15	<213> Artificial Sequence	
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20	<400> 20	
	ctaaaactca tacttaatag aagacaaacc	30
	<210> 21	
	<211> 41	
25	<212> DNA	
	<213> Artificial Sequence	
	<220>	
30	<223> Designed oligonucleotide primer to obtain raffinose synthase gene, n is i.	
	<400> 21	
	cgatggatgg gnaanttnat ncancngan tggganatgt t	41
35	<210> 22	
	<211> 32	
	<212> DNA	
	<213> Artificial Sequence	

- 85 -

- <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i, r is a or g.
- 5 <400> 22
 ggccacatnt tnacnarncc natnggngcn aa 32
- <210> 23
 <211> 30
 10 <212> DNA
 <213> Artificial Sequence
- <220>
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 15 <400> 23
 tgttactagg cgaaacaaga gtagctctga 30
- <210> 24
 20 <211> 47
 <212> DNA
 <213> Artificial Sequence
- <220>
 25 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i.
- <400> 24
 cgaggnggnt gncncncngg nttngtnatn atngangang gntggca 47
 30
- <210> 25
 <211> 29
 <212> DNA
 <213> Artificial Sequence
 35
- <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i, y is t or c, r is a or g.

- 86 -

- <400> 25
 atytttrtcna cngcnarrtc ytccatngt 29
- 5 <210> 26
 <211> 38
 <212> DNA
 <213> Artificial Sequence
- 10 <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i, y is t or c.
- 15 <400> 26
 ggnacntant ggytncangg ntgnccanattg gtnccantg 38
 <210> 27
 <211> 32
 <212> DNA
 <213> Artificial Sequence
- 20 <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i, r is a or g.
- 25 <400> 27
 ggccacattt tncacnarrcc natnggngcn aa 32
 <210> 28
 <211> 26
 30 <212> DNA
 <213> Artificial Sequence
- 35 <220>
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- <400> 28
 atctatttgt catgacgatg atccga 26
 <210> 29

- 87 -

<211> 30
 <212> DNA
 <213> Artificial Sequence

5 <220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 29
ggccctcatt cccatattgg gatgatcctc 30

10

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$\langle 211 \rangle$	30
$\langle 212 \rangle$	DNA
$\langle 213 \rangle$	Artificial Sequence

15

<220>

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20 <400> 30
aagcatgcc aacatacaca tgctcaacag 30

	$\langle 210 \rangle$	31
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25	$\langle 212 \rangle$	DNA
	$\langle 213 \rangle$	Artificial Sequence

<220>
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 31
agaccg gggg aaagctttgg gg ttactact 30

	$\langle 210 \rangle$	32
35	$\langle 211 \rangle$	28
	$\langle 212 \rangle$	DNA
	$\langle 213 \rangle$	Artificial Sequence

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	<400> 32	
5	tggatgggaa actttataca ccctgact	28
	<210> 33	
	<211> 28	
	<212> DNA	
10	<213> Artificial Sequence	
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	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
	<400> 33	
15	gacatgttcc catctacaca cccttgtg	28
	<210> 34	
	<211> 30	
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20	<213> Artificial Sequence	
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	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
25	<400> 34	
	ccaatttatg ttagtgatgt tgttggcaag	30
	<210> 35	
	<211> 26	
30	<212> DNA	
	<213> Artificial Sequence	
	<220>	
	<223> Designed oligonucleotide primer to obtain raffinose synthase gene.	
35	<400> 35	
	tcgactccca gggtagaatt gtcac	26
	<210> 36	

- 89 -

- <211> 35
 <212> DNA
 <213> Artificial Sequence
- 5 <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i.
- <400> 36
 10 cgattnaang tntggtggac nacncantgg gtngg 35
- <210> 37
 <211> 38
 <212> DNA
 15 <213> Artificial Sequence
- <220>
 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,
 n is i, r is a or g.
- 20 <400> 37
 cantgnacca tntgncancc ntgnarccan tangtncc 38
- <210> 38
 25 <211> 30
 <212> DNA
 <213> Artificial Sequence
- <220>
 30 <223> Designed oligonucleotide primer to obtain raffinose synthase gene.
- <400> 38
 gttagggttc atatgaacac cttcaagctc 30
- 35 <210> 39
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

26

10 <213> Artificial Sequence

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

30

⟨213⟩ Artificial Sequence

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

27

⟨213⟩ Artificial Sequence

35 <223> Designed oligonucleotide primer to obtain raffinose synthase gene.

30

- 91 -

<210> 43

<211> 30

<212> DNA

⟨213⟩ Artificial Sequence

5

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 43

10 gttagggttc atatgaacac cttcaagctc

30

<210> 44

<211> 29

<212> DNA

15 <213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

20 $\langle 400 \rangle$ 44

tctacgtctg gcacgcgctt tgcggctac

29

<210> 45

<211> 31

25 <212> DNA

⟨213⟩ Artificial Sequence

$\langle 220 \rangle$

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

30

<400> 45

ggtgacgtca tccacatatt ggagatgttg t

31

<210> 46

35 $\langle 211 \rangle$ 29

<212> DNA

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 $\langle 220 \rangle$

<400> 46

29

5

<211> 35

⟨213⟩ Artificial Sequence

10

 $\langle 220 \rangle$

<400> 47

15

35

<210> 48

<211> 25

<212> DNA

20

⟨213⟩ Artificial Sequence

 $\langle 220 \rangle$

25

<400> 248

25

<210> 49

<211> 25

30

<212> DNA

<213> Artificial Sequence

<220>

35

<400> 49

25

<210> 50

- 93 -

<211> 17
 <212> DNA
 <213> Artificial Sequence

5 <220>
 <223> Designed oligonucleotide linker to obtain raffinose synthase gene.

 <400> 50
 ggatccgaca cgagctc 17

10 <210> 51
 <211> 30
 <212> DNA
 <213> Artificial Sequence

15 <220>
 <223> Designed oligonucleotide primer to confirm direction of the inserted
 raffinose synthase gene.

20 <400> 51
 cctcctcgga ttccattgcc cagctatctg 30

 <210> 52
 <211> 30

25 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Designed oligonucleotide primer to confirm direction of the inserted
 30 raffinose synthase gene.

 <400> 52
 ggattcgaca caaaccgcca cgtcacgctc 30

35 <210> 53
 <211> 29
 <212> DNA
 <213> Artificial Sequence

- 94 -

<220>

<223> Designed oligonucleotide primer to confirm direction of the inserted
raffinose synthase gene.

5

<400> 53

tctacgtctg gcacgcgctt tgcggctac

29

- 95 -

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises a nucleotide sequence hybridizable with a nucleotide sequence complementary to a nucleotide sequence consisting of:

(a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5;

(b) the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6;

(c) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7; or

(d) the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8;

under stringent conditions of 6xSSC, 0.1% SDS at 65 to 68°C and encoding a protein being capable of binding D-galactosyl group through $\alpha(1\rightarrow6)$ bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose.

2. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5.

3. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6.

- 96 -

4. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7.
5. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8.
6. A polynucleotide encoding a raffinose synthase, wherein the polynucleotide comprises the nucleotide sequence represented by SEQ ID NO: 6 or SEQ ID NO: 8.
7. A nucleic acid comprising the polynucleotide as defined in any one of claims 1 to 6 which is joined to a nucleic acid exhibiting promoter activity in a host cell.
8. A vector comprising the polynucleotide as defined in any one of claims 1 to 6.
9. A cell transformed with the polynucleotide as defined in any one of claims 1 to 6.
10. A cell transformed with the nucleic acid as defined in claim 7.
11. A cell transformed with the vector as defined in claim 8.
12. The cell of any one of claims 9 to 11, wherein the cell is a microorganism cell.
13. The cell of any one of claims 9 to 11, wherein the cell is a plant cell.

- 97 -

14. A method for producing a raffinose synthase which comprises the steps of:

culturing or growing the cell as defined in any one of claims 9 to 13 to produce the raffinose synthase; and
collecting the raffinose synthase.

15. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 5.

16. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 7.