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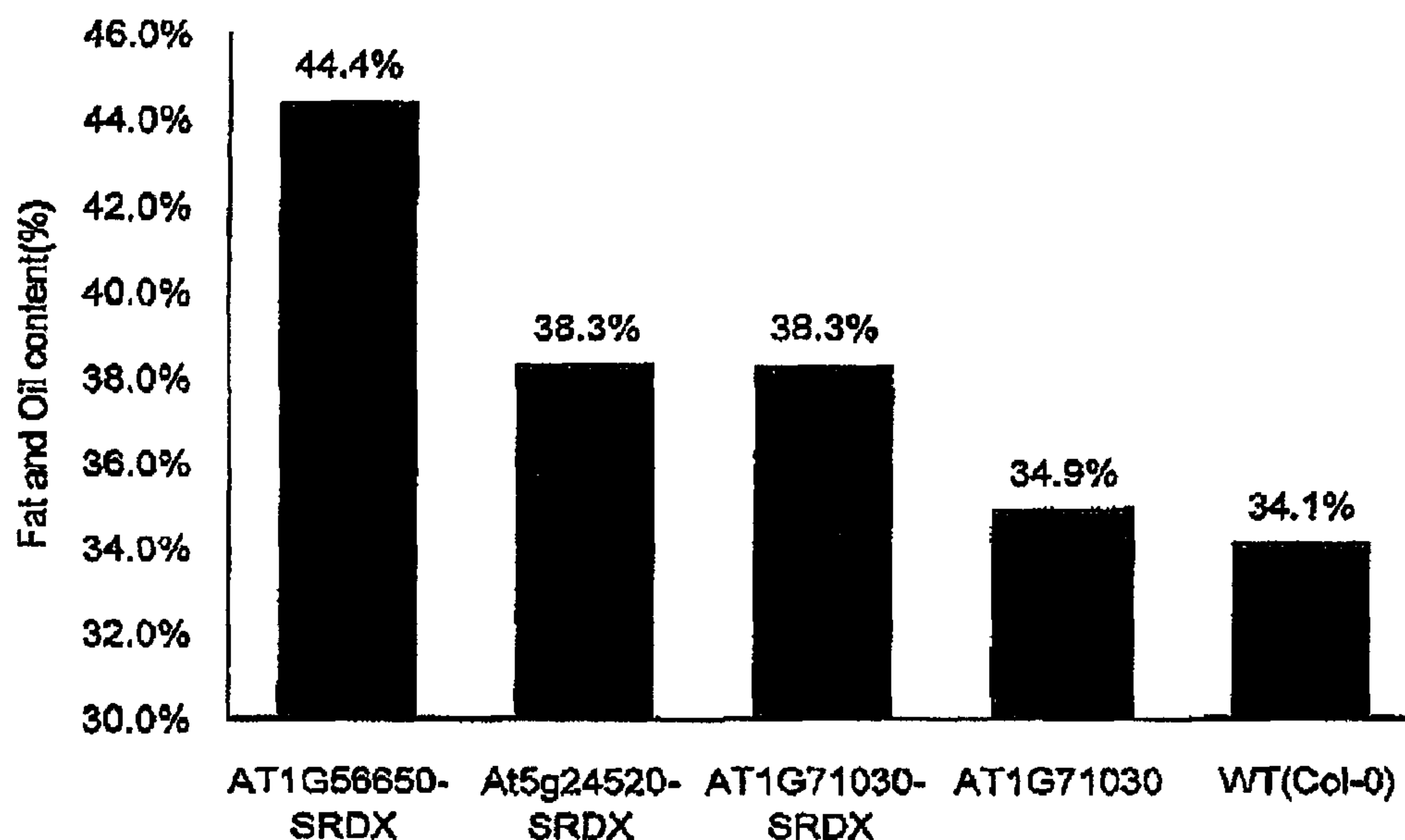
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(54) **Titre : GENES QUI AUGMENTENT LA PRODUCTION D'HUILE DANS LES PLANTES ET PROCEDE D'UTILISATION DE CES GENES**

(54) **Title: GENE THAT INCREASES PRODUCTION OF PLANT FAT-AND-OIL AND METHOD FOR USING THE SAME**



(57) **Abrégé/Abstract:**

This invention is intended to search for a transcription regulator having a novel function of improving the amount of substances produced per individual plant and to enhance such properties in a plant. A chimeric protein resulting from the fusion of a



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(57) **Abrégé(suite)/Abstract(continued):**

transcription factor belonging a transcription factor family that includes a transcription factor comprising the amino acid sequence as shown in SEQ ID NO: 4 with the repressor domain is expressed in a plant.

ABSTRACT

This invention is intended to search for a transcription regulator having a novel function of improving the amount of substances produced per individual plant and to enhance such properties in a plant. A chimeric protein resulting from the fusion of a transcription factor belonging a transcription factor family that includes a transcription factor comprising the amino acid sequence as shown in SEQ ID NO: 4 with the repressor domain is expressed in a plant.

DESCRIPTION

GENE THAT INCREASES PRODUCTION OF PLANT FAT-AND-OIL AND METHOD FOR USING THE SAME

Background Art

The term "biomass" generally refers to the total amount of organic material that inhabits or exists in a given area. When such term is used for plants, in particular, it refers to dry weight per unit area. A biomass unit is quantified in terms of a mass or an energy amount. In the case of plant biomass, the term "standing crop" is occasionally used to represent "biomass." Since plant biomass is generated by fixing atmospheric carbon dioxide with the use of solar energy, it can be regarded as so-called "carbon-neutral energy." Accordingly, an increase in the amount of plant biomass is effective for global environmental protection, the prevention of global warming, and mitigation of greenhouse gas emissions. Thus, a technique for increasing the production of plant biomass is industrially significant.

Plants are cultivated for the purpose of using some tissue thereof (e.g., seeds, roots, leaves, or stems) or for the purpose of producing various materials, such as fat-and-oils. Examples of fat-and-oils produced from plants that have been heretofore known include soybean oil, sesame oil, olive oil, coconut oil, rice oil, cottonseed oil, sunflower oil, corn oil, safflower oil, palm oil, and rapeseed oil. Such fat-and-oils are extensively used for household and industrial applications. Also, fat-and-oils produced from plants are used as starting materials for biodiesel fuels or bioplastics, and the applicability thereof is increasing for alternative energy to petroleum.

Under such circumstances, it is necessary for the industrial success of the production of fat-and-oils using plants that the amount of production per unit of cultivation area be improved. If the number of cultivated plants is assumed to be constant per unit of cultivation area, an improvement in the amount of fat-and-oil production per plant is found to be necessary. When fat-and-oils are extracted from seeds obtained from plants, an improvement in the amount of fat-and-oil production per

plant can be achieved via techniques of, for example, improving the seed yield per plant or increasing the fat-and-oil content in seeds.

Techniques for increasing the amount of fat-and-oil production from plant seeds are broadly classified into techniques based on an improvement in cultivation methods and techniques based on the development of plant varieties that can increase the amount of fat-and-oil production. Techniques based on the development of plant varieties that can increase the amount of fat-and-oil production are roughly classified as conventional breeding techniques such as crossing and molecular breeding techniques involving gene recombination. As techniques for increasing the amount of fat-and-oil production via gene recombination, A) a method of modifying a system for synthesizing triacylglycerol (TAG) of seeds, which is a main component of plant fat-and-oils, and B) a method of modifying a regulator gene that regulates plant morphogenesis or metabolism and expression of genes associated therewith are known.

In method A) above, the amount of TAG synthesized from a sugar produced via photosynthesis can be increased by (1) enhancing synthesis activity from a fatty acid or glycerol (i.e., TAG components) from sugars or (2) reinforcing the reaction of synthesizing TAG from glycerol and a fatty acid. In this regard, the following techniques have been reported as techniques that use genetic engineering techniques. An example of (1) is a technique in which cytosolic acetyl-coenzyme A carboxylase (ACCase) of *Arabidopsis thaliana* is overexpressed in rapeseed plastids and the fat-and-oil content in seeds is improved by 5% (Plant Physiology, 1997, Vol. 11, pp. 75-81). An example of (2) is a technique of increasing fat-and-oil production via overexpression of diacylglycerol acyltransferase (DGAT), which transfers an acyl group to the sn-3 position of diacylglycerol (Plant Physiology, 2001, Vol. 126, pp. 861-874). It is reported that the fat-and-oil content and the seed weight are increased as the DGAT expression level increases, and the number of seeds per plant may be occasionally increased according to the method of Plant Physiology, 2001, Vol. 126, pp. 861-874. The fat-and-oil content in *Arabidopsis thaliana* seeds was increased by 46% and the fat-and-oil amount per plant was increased by a maximum of about 125% by such technique.

As a form of method B), expression of transcriptional factor genes associated

with the regulation of biosynthetic enzyme gene expression may be regulated. An example thereof can be found in WO 01/36597. WO 01/36597 employs a technique in which recombinant plants are prepared via exhaustive overexpression or knocking out of transcriptional factors and genes that enhance the fat-and-oil content in seeds are then selected. WO 01/36597 discloses that overexpression of ERF subfamily B-4 transcriptional factor genes results in a 23% increase in the fat-and-oil content in seeds. WO 01/36597, however, does not disclose an increase or decrease in fat-and-oil content per plant. Also, Plant J., 2004, 40, 575-585 discloses that the overexpression of WRINKLED1, which is a transcriptional factor having the AP2/EREB domain, improves the fat-and-oil content in seeds.

Although molecular breeding techniques as described above intended for the improvement of various traits have been developed, techniques for improving the yield involving increasing plant weight, increasing a given tissue, or improving the amount of target substances produced have not yet been put to practical use.

This is considered to be due to the following reasons. That is, truly adequate genes have not yet been discovered, and new recombinant varieties that are found effective at the test phase are unable to exhibit expected effects during the practical phase under a variety of natural environments. Also, many genes are associated with quantitative traits, such as increased plant weight, increased weight of a given tissue, or the amount of target substances produced, in various steps from those involving the control system to those involving the metabolic system, and it was difficult to discover and develop a truly adequate and useful gene that improves quantitative traits. In order to overcome such problems, the discovery of dramatically effective new genes and the development of genes exhibiting effects under practical environments, even if the effectiveness thereof is equivalent to that of existing genes, are necessary.

Disclosure of the Invention

Object to Be Attained by the Invention

Under given circumstances, it is an object of the present invention to search for a gene having a novel function of improving the amount of substances produced per

individual plant, and particularly of improving the fat-and-oil content in seeds. Also, it is another object to provide a technique that can improve such properties of plants.

Means for Attaining the Object

The present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered that expression of a chimeric protein resulting from the fusion of a transcription factor belonging a given transcription factor family with a functional peptide that converts an arbitrary transcription factor into a transcription repressor (hereafter it may be referred to as a "repressor domain") would improve a variety of quantitative traits, particularly the amount of substances produced per individual plant, and more particularly the amount of fat-and-oils produced. This has led to the completion of the present invention.

The plant of the present invention is obtained by expressing a chimeric protein resulting from the fusion of a transcription factor belonging a transcription factor family that includes a protein comprising the amino acid sequence as shown in SEQ ID NO: 4 with a functional peptide that converts an arbitrary transcription factor into a transcription repressor. In the plant of the present invention, transcription regulating activity, and particularly transcription accelerating activity, is preferably suppressed in a given transcription factor via fusion of a functional peptide.

The transcription factor that fuses the above functional peptide is preferably any of proteins (a) to (c) below:

- (a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 4;
- (b) a protein comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 4 by deletion, substitution, addition, or insertion of 1 or a plurality of amino acids and having transcription accelerating activity; or
- (c) a protein encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence as shown in SEQ ID NO: 3 and having transcription accelerating activity.

Examples of the functional peptides include peptides represented by formulae (1) to (8) below:

(1) X1-Leu-Asp-Leu-X2-Leu-X3

wherein X1 represents 0 to 10 amino acid residues; X2 represents Asn or Glu; and X3 represents at least 6 amino acid residues;

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3

wherein Y1 represents 0 to 10 amino acid residues; Y2 represents Phe or Ile; and Y3 represents at least 6 amino acid residues;

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3

wherein Z1 represents Leu, Asp-Leu, or Leu-Asp-Leu; Z2 represents Glu, Gln, or Asp; and Z3 represents 0 to 10 amino acid residues;

(4) Asp-Leu-Z4-Leu-Arg-Leu

wherein Z4 represents Glu, Gln, or Asp;

(5) α 1-Leu- β 1-Leu- γ 1-Leu;(6) α 1-Leu- β 1-Leu- γ 2-Leu;(7) α 1-Leu- β 2-Leu-Arg-Leu; and(8) α 2-Leu- β 1-Leu-Arg-Leu

wherein, in formulae (5) to (8), α 1 represents Asp, Asn, Glu, Gln, Thr, or Ser; α 2 represents Asn, Glu, Gln, Thr, or Ser; β 1 represents Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His; β 2 represents Asn, Arg, Thr, Ser, or His; γ 1 represents Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp; and γ 2 represents Gln, Asn, Thr, Ser, His, Lys, or Asp.

A method for producing a substance with the use of the plant of the present invention comprises a step of separating and recovering a substance that can be produced in greater amounts from the plant of the present invention described above. An example of such substance is a fat-and-oil.

Further, the present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered that the amount of substances produced per individual plant, and particularly the amount of fat-and-oils produced, could be improved in a strain that defects a gene involving the pigment synthesis pathway, thereby completing the present invention. Examples of genes involving the pigment synthesis pathway include a gene encoding a factor associated with transportation of a substrate or product of metabolic reactions in the pigment synthesis

pathway, a gene encoding an enzyme that catalyzes metabolic reactions in the pigment synthesis pathway, and a gene encoding an enzyme that catalyzes a reaction for preparing a locus for metabolic reactions in the pigment synthesis pathway. Further examples include genes that regulate expression of a gene encoding a factor associated with transportation of a substrate or product of metabolic reactions in the pigment synthesis pathway, a gene encoding an enzyme that catalyzes metabolic reactions in the pigment synthesis pathway, and a gene encoding an enzyme that catalyzes a reaction for preparing a locus for metabolic reactions in the pigment synthesis pathway.

Specifically, the method for producing plant-derived fat-and-oils of the present invention comprises a step of recovering fat-and-oil components from seeds extracted from plants lacking functions of at least one gene selected from the group consisting of the chalcone synthase gene, the chalcone isomerase gene, and the flavone-3-hydrase gene.

Also, a method for screening for a plant with enhanced fat-and-oil content of the present invention comprises a step of extracting seeds from a plant that is a target of evaluation regarding the fat-and-oil content in seeds, and a step of observing the seed-coat color of extracted seeds and determining that the fat-and-oil content in seeds is high when such color is closer to white.

Effects of the Invention

The plant of the present invention can produce greater amounts of substances per individual plant. With the use of the plant of the present invention, accordingly, the amount of target substances produced can be improved, and a target substance can be produced at low cost.

The method for producing plant-derived fat-and-oils of the present invention can improve the amount of fat-and-oils produced because of the significantly improved fat-and-oil content per unit amount of seeds of a plant that lacks functions of a given gene.

Further, the method for screening for a plant with enhanced fat-and-oil content of the present invention comprises evaluating the fat-and-oil content in seeds in a non-

destructive manner. Thus, screening can be rapidly and easily carried out with the use of a small number of seeds.

This description includes part or all of the contents as disclosed in the description and/or drawings of Japanese Patent Application No. 2008-054008, which is a priority document of the present application.

Brief Description of the Drawings

Fig. 1 is a characteristic diagram showing the results of measuring the fat-and-oil content in seeds of strains into which the modified transcription factor gene, improved transcription coactivator gene, or transcription factor gene had been introduced and those of wild-type strains.

Fig. 2 is a characteristic diagram showing the results of measuring the fat-and-oil content in seeds of strains lacking the pigment synthesis pathway and of wild-type strains.

Fig. 3 is a characteristic diagram showing the results of comparing the integrated value of R, G, and B values of seed-coat color determined with the use of image data with that of seeds of wild-type strains.

Best Modes for Carrying out the Invention

Hereafter, the present invention is described in detail.

The plant of the present invention expresses a chimeric protein resulting from the fusion of a transcription regulator belonging a given transcription regulator family, particularly a transcription factor belonging a given transcription factor family, with a functional peptide that converts an arbitrary transcription factor into a transcription repressor, which can produce greater amounts of substances per individual plant compared with a wild-type plant. Specifically, the plant of the present invention expresses a chimeric protein of a transcription factor with the functional peptide, so as to significantly improve the amount of substances produced of a plant of interest.

In the plant of the present invention, in particular, the transcription accelerating

activity according to a transcription factor is preferably suppressed via fusion with the functional peptide. In other words, it is preferable in the plant of the present invention for expression of a chimeric protein resulting from the fusion of a transcription factor with the functional peptide to result in the appearance of the transcription repression effects caused by the functional peptide as dominant traits.

The term "the amount of substances produced per individual plant" used herein refers to the contents of various substances generated from plants per unit volume. Substances are not particularly limited and may be naturally produced by plants. Alternatively, such substances may be not naturally produced by plants, but rather may be produced from plants via genetic engineering or other means. If the content of the target product per tissue is increased, in particular, purification and transportation costs can be reduced, and the industrial usefulness of such plants is significant. Specifically, target products may be lignocelluloses that account for substantially the entire weight of a plant, or plant oils that are used as seed oils at the industrial level may be used. Plant oil may be a simple lipid as an ester of fatty acid with alcohol, a complex lipid including phosphorus, sugar, nitrogen, and the like, or a fatty acid. An alcohol of a simple lipid may be a higher alcohol having a high molecular weight or a polyhydric alcohol, such as glycerol (glycerin). A fatty acid of a simple lipid may be a saturated fatty acid, unsaturated fatty acid, or special fatty acid comprising a hydroxyl group or an epoxy group. A simple lipid as the ester of glycerol and fatty acid may be monoacylglycerol, diacylglycerol, or triacylglycerol.

Hereafter, substances that can be produced in greater amounts are described with reference to fat-and-oils, although the technical scope of the present invention is not limited thereto. The present invention is also applicable to substances other than fat-and-oils as substances generated from plants.

Any plant can be the target of the present invention without particular limitation. Plants that have been heretofore used for the production of fat-and-oils are particularly preferable. Examples of plants that can serve as targets include soybeans, sesame, olive oils, coconuts, rice, cottons, sunflowers, maize, sugar cane, *Jatropha*, oil palm, tobacco, safflowers, and rapeseeds. Also, *Arabidopsis thaliana*, which is

extensively used as a model organism in genetic analysis of plants and for which a method for gene expression analysis has been established can be a target plant.

Transcription repression activity according to a chimeric protein of a transcription factor refers to actively repressing expression of a gene located downstream by recognizing a cis sequence that is recognized by the transcription factor or recognizing another cis sequence recognized by other transcription factor similar to the former cis sequence. It may be referred to as a "transcription repressor." A method for repressing a transcription that is an activity according to the chimeric protein of the transcription factor is not particularly limited, but, most preferably, is a method for constructing a chimeric protein comprising a repressor domain sequence or an SRDX sequence (i.e., a fusion protein) is most preferable.

In such method, the term "repressor domain sequence" refers to an amino acid sequence constituting a peptide that converts an arbitrary transcription factor into a transcription repressor, and the present inventors have discovered various types of such sequence. Techniques involving the use of repressor domain sequences are disclosed in, for example, JP Patent Publication (kokai) No. 2001-269177 A, JP Patent Publication (kokai) No. 2001-269178 A, JP Patent Publication (kokai) No. 2001-292776 A, JP Patent Publication (kokai) No. 2001-292777 A, JP Patent Publication (kokai) No. 2001-269176 A, JP Patent Publication (kokai) No. 2001-269179 A, WO 03/055903, Ohta, M., Matsui, K., Hiratsu, K., Shinshi, H. and Ohme-Takagi, M., *The Plant Cell*, Vol. 13, 1959-1968, August, 2001, and Hiratsu, K., Ohta, M., Matsui, K., Ohme-Takagi, M., *FEBS Letters* 514, 2002, 351-354. Repressor domain sequences are cleaved from Class II ethylene-responsive element binding factor (ERF) proteins or plant zinc finger proteins (e.g., the *Arabidopsis thaliana* SUPERMAN protein) and have very simple structures.

An example of a transcription regulator expressed as a chimeric protein is the *Arabidopsis thaliana* transcription factor identified with the AGI code At1g71030 (hereafter simply referred to as the "transcription factor At1g71030"). The transcription factor At1g71030 is a transcription factor belonging the myb family, which is known to be similar to MybHv5 GI:19055 derived from barley. SEQ ID NO: 4

shows the amino acid sequence of the transcription factor At1g71030. SEQ ID NO: 3 shows the nucleotide sequence of the gene encoding the transcription factor At1g71030.

In addition, the transcription coactivator identified as At5g24520 (hereafter simply referred to as the "transcription coactivator At5g24520"), the transcription repressor, and the transcription corepressor are known as transcription regulators. A chimeric protein of such transcription coactivator or transcription repressor comprising a repressor domain can also be constructed. The factor identified with the AGI code At5g24520 is a transcription coactivator known as the transparent testa glabra 1 protein (TTG1). Regarding genes derived from other plants, a protein encoded by the *Malus domestica*-derived GenBank Accession Number AAF27919, a protein encoded by the *Petunia hybrida*-derived GenBank Accession Number AAC18914, a protein encoded by the *Gossypium hirsutum*-derived GenBank Accession Number AAM95645, and a protein encoded by the *Perilla frutescens*-derived GenBank Accession Number BAB58883 are known to be homologous to the transcription coactivator At5g24520, and such proteins are expected to exhibit functions equivalent to those described herein. SEQ ID NO: 2 shows the amino acid sequence of the transcription coactivator At5g24520. SEQ ID NO: 1 shows the nucleotide sequence of the gene encoding the transcription coactivator At5g24520.

The transcription coactivator At5g24520 and the transcription factor At1g71030, chimeric proteins of which are to be prepared, are not limited to those comprising the amino acid sequences as shown in SEQ ID NOs: 2 and 4. A transcription coactivator or transcription factor comprising an amino acid sequence derived from the amino acid sequence as shown in SEQ ID NO: 2 or 4 by deletion, substitution, addition, or insertion of 1 or a plurality of amino acids and having transcription accelerating activity may be used. The number of such plurality of amino acids is, for example, 1 to 20, preferably 1 to 10, more preferably 1 to 7, further preferably 1 to 5, and particularly preferably 1 to 3. Deletion, substitution, or addition of amino acids can be realized by modifying a nucleotide sequence encoding the above-mentioned transcription factor via a method known in the art. Mutation can be introduced into a nucleotide sequence via known methods, such as the Kunkel or

Gapped duplex method, or methods in accordance therewith. For example, mutation is introduced with the use of mutagenesis kits utilizing site-directed mutagenesis (e.g., Mutant-K or Mutant-G (tradenames, manufactured by Takara Bio Inc.)) or the LA PCR *In vitro* Mutagenesis Series Kit (tradename, manufactured by Takara Bio Inc.). A mutation may be introduced via a method involving the use of chemical mutagens typified by EMS (ethyl methane sulfonate), 5-bromouracil, 2-aminopurine, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine, or other carcinogenic compounds, radiation processing typified by the use of x-rays, alpha rays, beta rays, gamma rays, or ion beams, or ultrasonic processing.

Further, transcription coactivators and transcription factors, chimeric proteins of which are to be prepared, are not limited to *Arabidopsis thaliana* transcription coactivator At5g24520 and transcription factor At1g71030. Transcription coactivators and transcription factors having equivalent functions in plants other than *Arabidopsis thaliana* (e.g., plants mentioned above) are included (hereafter referred to as "homologous transcription coactivator(s)" or "homologous transcription factor(s)"). Transcription coactivators homologous to the transcription coactivator At5g24520 or transcription factors homologous to the transcription factor At1g71030 can be searched for using the genome information of a target plant based on the amino acid sequences of the transcription coactivator At5g24520 or the transcription factor At1g71030 or the nucleotide sequences of such genes, if the plant genome information has been revealed. As a homologous coactivator and a transcription factor, an amino acid sequence having, for example, 70% or higher, preferably 80% or higher, more preferably 90% or higher, and most preferably 95% or higher homology to the amino acid sequence of the transcription coactivator At5g24520 or the transcription factor At1g71030 is searched for. Homology values are determined by default using a computer program that implements the BLAST algorithm and a database that stores gene sequence information.

When the plant genome information has not been revealed, the genome is extracted from the target plant, or a cDNA library of the target plant is constructed. The genome region or cDNA hybridizing under stringent conditions to at least part of the nucleotide sequence of the transcription coactivator At5g24520 or the transcription

factor At1g71030 is then isolated. Thus, a homologous gene can be identified. Under stringent conditions, a so-called specific hybrid is formed, but a non-specific hybrid is not formed. For example, hybridization is carried out at 45°C in the presence of 6× SSC (sodium chloride/sodium citrate), followed by washing at 50°C to 60°C in the presence of 0.2 to 1× SSC and 0.1% SDS. Alternatively, hybridization is carried out at 65°C to 70°C in the presence of 1× SSC, followed by washing at 65°C to 70°C in the presence of 0.3× SSC. Hybridization can be carried out in accordance with a conventional technique, such as the method described in J. Sambrook et al. *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, 1989.

The plant of the present invention significantly improves the amount of fat-and-oil production via expression of a chimeric protein of a transcription factor with a functional peptide. Also, the plant of the present invention significantly improves the amount of fat-and-oil production when the chimeric protein of a transcription coactivator with a functional peptide is expressed. By expressing a chimeric protein, in particular, a target transcription factor is expressed while transcription accelerating activity is suppressed, transcription repressing activity for recognizing a cis sequence homologous to the cis sequence that is recognized by the target transcription factor is expressed, and affinity and specificity to other factors, nucleic acids, lipids, or saccharides of the target transcription factor and transcription coactivator is changed, and the amount of fat-and-oil production is thus significantly improved. In such plant, the endogenous transcription factor or transcription coactivator may be modified to prepare a chimeric protein thereof. Alternatively, a gene encoding a chimeric protein may be introduced and such gene may be expressed.

A preferable example of such technique is a technique comprising introducing a gene encoding a chimeric protein (i.e., a fusion protein) resulting from the fusion of the aforementioned transcription factor or transcription coactivator with a functional peptide that converts an arbitrary transcription factor into a transcription repressor and expressing such chimeric protein (i.e., fusion protein) therein.

The term "transcription factor with suppressed transcription accelerating activity" used herein refers to a transcription factor exhibiting significantly lower

transcription accelerating activity than the transcription factor originally had, without particular limitation. Also, the term "a functional peptide that converts an arbitrary transcription factor into a transcription repressor" refers to a peptide that, when it is fused with an arbitrary transcription factor and converted into a chimeric protein, has a function of significantly lowering the transcription accelerating activity to a level below that the transcription factor originally had. (It may be occasionally referred to as a "transcription repressor converting peptide".) A "functional peptide that converts an arbitrary transcription factor into a transcription repressor" used herein is not particularly limited, and it is particularly preferable for a peptide to comprise an amino acid sequence known as a repressor domain sequence or SRDX sequence. Such transcription repressor converting peptide is described in detail in JP Patent Publication (kokai) No. 2005-204657 A, and all peptides disclosed therein can be used.

Examples of transcription repressor converting peptides include amino acid sequences represented by formulae (1) to (8) below:

(1) X1-Leu-Asp-Leu-X2-Leu-X3

wherein X1 represents 0 to 10 amino acid residues; X2 represents Asn or Glu; and X3 represents at least 6 amino acid residues;

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3

wherein Y1 represents 0 to 10 amino acid residues; Y2 represents Phe or Ile; and Y3 represents at least 6 amino acid residues;

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3

wherein Z1 represents Leu, Asp-Leu, or Leu-Asp-Leu; Z2 represents Glu, Gln, or Asp; and Z3 represents 0 to 10 amino acid residues;

(4) Asp-Leu-Z4-Leu-Arg-Leu

wherein Z4 represents Glu, Gln, or Asp;

(5) α 1-Leu- β 1-Leu- γ 1-Leu;

(6) α 1-Leu- β 1-Leu- γ 2-Leu;

(7) α 1-Leu- β 2-Leu-Arg-Leu; and

(8) α 2-Leu- β 1-Leu-Arg-Leu

wherein, in formulae (5) to (8), α 1 represents Asp, Asn, Glu, Gln, Thr, or Ser; α 2

represents Asn, Glu, Gln, Thr, or Ser; $\beta 1$ represents Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His; $\beta 2$ represents Asn, Arg, Thr, Ser, or His; $\gamma 1$ represents Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp; and $\gamma 2$ represents Gln, Asn, Thr, Ser, His, Lys, or Asp.

Transcription repressor converting peptide represented by formula (1)

The number of amino acid residues represented by X1 of the transcription repressor converting peptide represented by formula (1) may be 0 to 10. Specific types of amino acids that constitute the amino acid residues represented by X1 are not particularly limited, and any amino acid may be used. It is preferable that the number of amino acid residues represented by X1 be as small as possible from the viewpoint of ease of synthesis of the transcription repressor converting peptide represented by formula (1). Specifically, the number of amino acid residues represented by X1 is preferably 5 or less.

Also, the number of the amino acid residues represented by X3 of the transcription repressor converting peptide represented by formula (1) may be at least 6. Specific types of amino acids that constitute the amino acid residues represented by X3 are not particularly limited, and any amino acid may be used.

Transcription repressor converting peptide represented by formula (2)

The number of the amino acid residues represented by Y1 of the transcription repressor converting peptide represented by formula (2) may be 0 to 10, as in the case of X1 of the transcription repressor converting peptide represented by formula (1). Also, specific types of amino acids that constitute the amino acid residues represented by Y1 are not particularly limited, and any amino acid may be used. Specifically, the number of amino acid residues represented by Y1 is preferably 5 or less.

Also, the number of the amino acid residues represented by Y3 of the transcription repressor converting peptide represented by formula (2) may be at least 6, as in the case of X3 of the transcription repressor converting peptide represented by formula (1). Also, specific types of amino acids that constitute the amino acid residues represented by Y3 are not particularly limited, and any amino acid may be used.

Transcription repressor converting peptide represented by formula (3)

The amino acid residues represented by Z1 of the transcription repressor

converting peptide represented by formula (3) comprise 1 to 3 Leu residues: i.e., Leu when the number of amino acids is 1; Asp-Leu when the number of amino acids is 2; and Leu-Asp-Leu when the number of amino acids is 3.

In contrast, the number of the amino acid residues represented by Z3 of the transcription repressor converting peptide represented by formula (3) may be 0 to 10. Also, specific types of amino acids that constitute the amino acid residues represented by Z3 are not particularly limited, and any amino acid may be used. Specifically, the number of amino acid residues represented by Z3 is preferably 5 or less. Specific examples of amino acid residues represented by Z3 include, but are not limited to, Gly, Gly-Phe-Phe, Gly-Phe-Ala, Gly-Tyr-Tyr, and Ala-Ala-Ala.

The number of amino acid residues constituting the entire transcription repressor converting peptide represented by formula (3) is not particularly limited. From the viewpoint of ease of synthesis, the number of amino acids is preferably 20 or less.

Transcription repressor converting peptide represented by formula (4)

The transcription repressor converting peptide represented by formula (4) is a hexamer (6-mer) comprising 6 amino acid residues. When the amino acid residue represented by Z4 of the transcription repressor converting peptide represented by formula (4) is Glu, the amino acid sequence of interest is equivalent to the amino acid sequence composed of amino acids 196 to 201 of the *Arabidopsis thaliana* SUPERMAN protein (SUP protein).

Various types of transcription repressor converting peptides described above are fused with the aforementioned transcription factors or transcription coactivators to obtain chimeric proteins (i.e., fusion proteins), so that properties of such transcription factors or transcription coactivators can be modified. Specifically, the transcription repressor converting peptides may be fused with the aforementioned transcription factors or transcription coactivators to obtain chimeric proteins (i.e., fusion proteins), so that such transcription factors or transcription coactivators can be modified in the form of transcription repressors or negative transcription coactivators. Further, non-dominant transcription repressors can be modified in the form of dominant transcription

repressors.

In addition, a fusion gene of a polynucleotide encoding the transcription repressor converting peptide and a gene encoding a transcription factor or transcription coactivator may be produced to obtain a chimeric protein (i.e., a fusion protein). Specifically, a polynucleotide encoding the transcription repressor converting peptide (referred to as a "transcription repressor converting polynucleotide") is ligated to a gene encoding the transcription factor or transcription coactivator to construct a fusion gene, and the resultant is then introduced into a plant cell. Thus, chimeric proteins (i.e., fusion proteins) can be produced. Specific nucleotide sequences of the transcription repressor converting polynucleotides are not particularly limited, and such polynucleotides may comprise nucleotide sequences corresponding to the amino acid sequences of the transcription repressor converting peptides based on genetic code. The transcription repressor converting polynucleotides may comprise nucleotide sequences that serve as ligation sites to be connected to the transcription factor genes, according to need. When the amino acid reading frame of the transcription repressor converting polynucleotide is not aligned with that of the transcription factor or coactivator gene, further, the polynucleotide may comprise an additional nucleotide sequence, so as to align the reading frames. Further, the polynucleotide may comprise various additional polypeptides, such as a polypeptide having a linker function for connecting the transcription factor or transcription coactivator to the transcription repressor converting peptide or a polypeptide for labeling the chimeric protein (i.e., fusion protein) with an epitope, such as His, Myc, or Flag. Further, the chimeric protein (i.e., fusion protein) may comprise a structure other than a polypeptide, such as a sugar chain or an isoprenoid group, according to need.

The method for producing the plant is not particularly limited, provided that the method comprises a step of producing a chimeric protein of the transcription factor or transcription coactivator with a transcription repressor converting peptide in a plant to improve the amount of fat-and-oils produced. An example thereof is a production method comprising steps of construction of an expression vector, transformation, and selection. Such steps are described in detail below.

Step of constructing expression vector

A step of constructing an expression vector is not particularly limited, provided that a recombinant expression vector comprising the gene encoding the above-mentioned transcription factor or transcription coactivator, the transcription repressor converting polynucleotide, and a promoter is constructed. A variety of known vectors can be used as matrices for recombinant expression vectors. Examples of vectors that can be used include plasmid, phage, and cosmid vectors, and adequate vectors can be selected in accordance with the plant cells to which such vectors are introduced or methods of introduction into a cell. Specific examples include pBR322, pBR325, pUC19, pUC119, pBluescript, pBluescriptSK, and pBI vectors. When a vector is introduced into a plant by the *Agrobacterium* method, in particular, use of the pBI binary vector is preferable. Specific examples of pBI binary vectors include pBIG, pBIN19, pBI101, pBI121, and pBI221 vectors.

Promoters are not particularly limited, provided that such promoters can express a gene of interest in a plant. Known promoters are preferably used. Examples of such promoters include cauliflower mosaic virus 35S promoters (CaMV 35S), actin gene promoters, ubiquitin gene promoters, noparin synthase gene promoters, tobacco PR1a gene promoters, and ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit promoters, napin gene promoters, and oleosin gene promoters in tomatoes. Among such promoters, cauliflower mosaic virus 35S promoters, actin gene promoters, and ubiquitin gene promoters are preferable. With the use of such promoters, arbitrary genes can be intensively expressed upon introduction of the resulting recombinant expression vector into plant cells. A promoter is ligated so as to express the fusion gene of the gene encoding the transcription factor or transcription coactivator and the transcription repressor converting polynucleotide, and the resultant may be introduced into the vector in that state. The specific structure of a recombinant expression vector is not particularly limited.

The recombinant expression vector may further comprise other DNA segments, in addition to the promoter and the fusion gene. Such other DNA segments are not particularly limited, and examples thereof include a terminator, a selection marker, an

enhancer, and a nucleotide sequence for enhancing translation efficiency. Also, the recombinant expression vector may further comprise a T-DNA region. The T-DNA region can enhance the efficiency of gene introduction, particularly when introducing the recombinant expression vector into a plant with the use of *Agrobacterium*.

A transcription terminator is not particularly limited, provided that it functions as a transcription termination site, and a known terminator may be used. Specific examples of transcription terminators that can be preferably used include the transcription termination region of the nopal synthase gene (the Nos terminator) and the transcription termination region of the cauliflower mosaic virus 35S (the CaMV 35S terminator), with the Nos terminator being more preferable. The recombinant vector can be used to avoid the occurrence of phenomena such as synthesis of an unnecessarily long transcript after the introduction thereof into plant cells or a reduction in the plasmid copy number caused by a potent promoter by positioning a transcription terminator in an adequate site.

Drug-resistance genes can be used as selection markers for transformants, for example. Specific examples of such drug-resistance genes include drug-resistance genes that are resistant to hygromycin, bleomycin, kanamycin, gentamicin, and chloramphenicol. Plants that grow in a medium containing the above antibiotics may be selected with the use of such selection markers, so that transformed plants can be easily selected.

An example of a nucleotide sequence for enhancing translation efficiency is the omega sequence derived from the tobacco mosaic virus. This omega sequence may be located in the untranslated region (5' UTR) of the promoter to enhance the translation efficiency of the fusion gene. Thus, the recombinant expression vector can comprise a variety of DNA segments in accordance with its intended purposes.

Methods for constructing recombinant expression vectors are not particularly limited. The promoter, the gene encoding the transcription factor or transcription coactivator, the transcription repressor converting polynucleotide, and, according to need, other DNA segments may be introduced into an adequately selected matrix vector in a predetermined order. For example, the gene encoding the transcription factor may

be ligated to the transcription repressor converting polynucleotide to construct a fusion gene, the fusion gene may then be ligated to the promoter (e.g., a transcription terminator according to need) to construct an expression cassette, and the resulting expression cassette may be introduced into the vector.

When constructing a chimeric gene (fusion gene) and an expression cassette, for example, cleavage sites of DNA segments are made to be protruding ends that are complementary to each other, such DNA segments are subjected to a reaction with the aid of ligation enzymes, and the order of such DNA segments can be determined. When an expression cassette comprises a terminator, the expression cassette may comprise the promoter, the chimeric gene, and the terminator, in that order from upstream. Also, the types of reagents used for constructing a recombinant expression vector (i.e., restriction enzymes or ligation enzymes) are not particularly limited, and commercially available products may be adequately selected and used.

Also, methods for growing the recombinant expression vector (i.e., methods of production) are not particularly limited, and known methods can be employed. In general, *E. coli* hosts may be used, and the recombinant expression vector may be grown therein. In such a case, preferable *E. coli* species may be selected in accordance with vector type.

Step of transformation

The step of transformation that is carried out in the present invention comprises introducing the aforementioned fusion genes into a plant cell so as to express such genes therein with the aid of the recombinant expression vector. Methods of gene introduction into a plant cell with the aid of a recombinant expression vector (i.e., methods of transformation) are not particularly limited, and adequate known methods can be employed in accordance with a given plant cell. Specific examples of such methods include a method involving the use of *Agrobacterium* and a method involving direct introduction of a gene into a plant cell. Examples of methods involving the use of *Agrobacterium* that can be employed include methods described in Bechtold, E., Ellis, J., and Pelletier, G., 1993, *In Planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis plants*, C. R. Acad. Sci. Paris Sci. Vie, 316, 1194-1199

and Zyprian E., Kado C.L., *Agrobacterium*-mediated plant transformation by novel mini-T vectors in conjunction with a high-copy vir region helper plasmid, *Plant Molecular Biology*, 1990, 15 (2), 245-256.

Examples of methods involving direct introduction of a recombinant expression vector with DNA comprising the target gene into a plant cell include microinjection, electroporation, the polyethylene glycol method, the particle gun method, the protoplast fusion method, and the calcium phosphate method.

When the method involving direct introduction of DNA into a plant cell is employed, DNA comprising a transcription unit that is necessary for expression of a target gene, such as DNA comprising a promoter, a transcription terminator, and a target gene, is sufficient, and vector functions are not necessary. Also, DNA comprising a protein coding region of a target gene without a transcription unit may be used, provided that such DNA can be integrated into a host transcription unit and express the target gene therein.

Examples of plant cells into which DNA comprising the recombinant expression vector and the target gene or DNA comprising the target gene without the expression vector are to be introduced include tissue cells in plant organs such as flowers, leaves, and roots, calluses, and suspension cultured cells. According to the method for producing plants of the present invention, the recombinant expression vector may be adequately constructed in accordance with the type of plant to be produced. Alternatively, a general-purpose recombinant expression vector may be constructed in advance and it may be introduced into a plant cell. Specifically, the method for producing plants of the present invention may or may not comprise the step of constructing DNA used for transformation with the use of the recombinant expression vector.

Other steps and other methods

The method for producing the plant of the present invention may comprise the above-described method of transformation. Further, the method may comprise a step of constructing DNA used for transformation with the use of a recombinant expression vector and other steps. Specifically, the method may comprise a step of selecting

adequate transformants from transformed plants.

Methods of selection are not particularly limited. For example, transformants may be selected based on drug resistance, such as hygromycin-resistance, or based on the content of fat-and-oils in plants or arbitrary organs or tissues after the transformed plants have been grown. For example, transformants may be selected based on fat-and-oil content by quantifying the fat-and-oil components in seeds of the transformants in accordance with a conventional technique and comparing the quantified value with the fat-and-oil content in seeds of non-transformed plants (see the examples below).

According to the method for producing the plant of the present invention, the fusion gene is introduced into a plant. Thus, offspring plants exhibiting significantly improved fat-and-oil content can be obtained from such plant via sexual or asexual reproduction. Also, plant cells or reproductive materials, such as seeds, fruits, stocks, calluses, tubers, cut ears, or lumps, may be obtained from a plant or an offspring plant thereof, and a plant of interest can be mass-produced therefrom. The method for producing the plant of the present invention, accordingly, may comprise a step of growing the selected plant (i.e., a step of mass production).

The term "plant" used herein refers to a grown plant, a plant cell, a plant tissue, a callus, or a seed. According to the present invention, specifically, substances that can eventually grow into individual plants are regarded as plants. Plant cells can exist in various forms. Examples of such plant cells include suspension cultured cells, protoplasts, and leaf sections. Such plant cells may be grown and differentiated to obtain plants. Plants can be reproduced from plant cells via a known technique in accordance with plant cell type. The method for producing the plant of the present invention, accordingly, may comprise a step of reproducing plants from plant cells or the like.

The method for producing the plant of the present invention is not limited to a method in which transformation is carried out with the aid of a recombinant expression vector, and other methods may be employed. Specifically, a chimeric protein (i.e., a fusion protein) may be introduced into a plant, for example. In such a case, the chimeric protein (i.e., a fusion protein) may be introduced into a young plant so as to

improve the fat-and-oil content in a site of a plant that is to be eventually used. Methods for introducing the chimeric protein (i.e., a fusion protein) are not particularly limited, and various known methods may be employed.

As described above, the present invention can provide a plant that can produce greater amounts of substances per individual plant compared with that of a wild-type plant via expression of a chimeric protein of a transcription factor belonging a given transcription factor family with a functional peptide. The present invention can also provide a plant that can produce greater amounts of substances per individual plant compared with a wild-type plant via expression of a chimeric protein of a given transcription coactivator with a functional peptide. When such chimeric protein is expressed in a plant, transcription accelerating activity of a target transcription factor may occasionally be suppressed, or transcription suppressing effects may be exhibited on a sequence homologous to the cis sequence that is recognized by the target transcription factor. Further, a chimeric protein occasionally acts on other factors, DNA, RNA, lipids, or saccharides having affinity with the target transcription factor or transcription coactivator so as to alter such affinity and specificity. Also, a chimeric protein may occasionally act so as to improve affinity of a substance that has no affinity with the target transcription factor. In the plant of the present invention, the transcription factor that is subjected to a chimeric protein, a transcription factor that recognizes a cis sequence homologous to the cis sequence recognized by the aforementioned transcription factor, a transcription factor homologous to the aforementioned transcription factor that is subjected to a chimeric protein, other factors having affinity with the aforementioned transcription factor that is subjected to a chimeric protein, or the like is also expressed therein. However, expression of the target gene can be suppressed in a dominant-negative manner by the effects of the chimeric protein. This alters the expression levels of genes involved in fat-and-oil production and/or genes involved in decomposition of the produced fat-and-oils in the plant of the present invention. This is considered to result in the significantly enhanced fat-and-oil content.

The term "significantly enhanced fat-and-oil content" refers to a situation in

which an amount of the fat-and-oil has been enhanced, although seed mass per grain has not changed compared with wild-type plants, a situation in which an amount of the fat-and-oil has been enhanced with significantly increased seed mass per grain compared with wild-type plants, or a situation in which the fat-and-oil content in seeds is enhanced compared with wild-type plants. All cases indicate increased amounts of fat-and-oils produced by an individual plant. The plant of the present invention can be used for the method for producing plant-derived fat-and-oils. For example, the plant of the present invention is allowed to grow, seeds are collected, and fat-and-oil components are extracted from the collected seeds. Thus, fat-and-oils can be produced.

It can be said that the method for producing fat-and-oils utilizing the plant of the present invention is particularly excellent in terms of the amount of production because of the high fat-and-oil content in an individual plant. If the number of cultivated plants is assumed to be constant per unit of cultivation area, specifically, the amount of fat-and-oils produced per unit of cultivation area is significantly increased with the use of the plant of the present invention. With the use of the plant of the present invention, accordingly, production costs required for the production of fat-and-oils can be remarkably reduced.

Further, the method for producing fat-and-oils with the use of the plant of the present invention realizes high fat-and-oil content in seeds per unit weight. Thus, the method can be said to be excellent in terms of the amount of fat-and-oil production.

In the method for producing fat-and-oils using the plant of the present invention, fat-and-oils to be produced are not particularly limited. Examples thereof include plant-derived fat-and-oils, such as soybean oil, sesame oil, olive oil, coconut oil, rice oil, cottonseed oil, sunflower oil, corn oil, safflower oil, and rapeseed oil. The produced fat-and-oils can be extensively used for household or industrial applications. Further, such fat-and-oils can be used as starting materials for biodiesel fuels. With the use of the plant of the present invention, specifically, such fat-and-oils for household or industrial applications, biodiesel fuels, and the like can be produced at low cost.

Method for producing plant-derived fat-and-oils

According to the present invention, it was discovered that the fat-and-oil

content was significantly improved in seeds extracted from a plant exhibiting a certain type of phenotype. Specifically, seeds extracted from 4 types of pigment synthesis pathway-deficient strains (i.e., tt4, tt5, tt6, and Δ CHS strains) disclosed in a reference document (Plant J., Nov. 1995, 8 (5): 659-71) exhibit significantly improved fat-and-oil content in seeds compared with seeds extracted from wild-type plants. Specifically, the method for producing plant-derived fat-and-oils of the present invention comprises a step of recovering a fat-and-oil component from seeds extracted from a plant that lacks functions of at least 1 gene selected from the group consisting of the chalcone synthase gene, the chalcone isomerase gene, and the flavone-3-hydrase gene. The tt4 and the Δ CHS strains disclosed in the above document defect the chalcone synthase gene, the tt5 strain defects the chalcone isomerase gene, and the tt6 strain defects the flavone-3-hydrase gene.

SEQ ID NO: 5 shows the nucleotide sequence of the *Arabidopsis thaliana* chalcone synthase gene, and SEQ ID NO: 6 shows the amino acid sequence of chalcone synthase encoded by such gene. SEQ ID NO: 7 shows the nucleotide sequence of the *Arabidopsis thaliana* chalcone isomerase gene, and SEQ ID NO: 8 shows the amino acid sequence of chalcone isomerase encoded by such gene. SEQ ID NO: 9 shows the nucleotide sequence of the *Arabidopsis thaliana* flavone-3-hydrase gene, and SEQ ID NO: 10 shows the amino acid sequence of the flavone-3-hydrase encoded by such gene.

In the present invention, the chalcone synthase gene, the chalcone isomerase gene, and the flavone-3-hydrase gene are not limited to those comprising the sequences specifically described above. Specifically, the chalcone synthase gene, the chalcone isomerase gene, and the flavone-3-hydrase gene may each encode a protein comprising an amino acid sequence derived from the amino acid sequence specifically described above by deletion, substitution, addition, or insertion of 1 or a plurality of amino acids and having chalcone synthase activity, chalcone isomerase activity, and flavone-3-hydrase activity. The number of such plurality of amino acids is, for example, 1 to 20, preferably 1 to 10, more preferably 1 to 7, further preferably 1 to 5, and particularly preferably 1 to 3. Deletion, substitution, or addition of amino acids can be realized by modifying the nucleotide sequence specifically described above via a method known in

the art. Mutation can be introduced into a nucleotide sequence via known methods, such as the Kunkel or Gapped duplex method, or methods in accordance therewith. For example, mutation is introduced with the use of mutagenesis kits utilizing site-directed mutagenesis (e.g., Mutant-K or Mutant-G (tradenames, manufactured by Takara Bio Inc.)) or the LA PCR *in vitro* Mutagenesis Series Kit (tradename, manufactured by Takara Bio Inc.). A mutation may be introduced via a method involving the use of chemical mutagens typified by EMS (ethyl methane sulfonate), 5-bromouracil, 2-aminopurine, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine, or other carcinogenic compounds, radiation processing typified by the use of x-rays, alpha rays, beta rays, gamma rays, or ion beams, or ultrasonic processing.

In the present invention, the chalcone synthase gene, the chalcone isomerase gene, and the flavone-3-hydrase gene further comprise genes having equivalent functions in plants other than *Arabidopsis thaliana* (e.g., plants described above) (hereafter such genes are referred to as "homologous genes"). Homologous genes of the chalcone synthase gene, the chalcone isomerase gene, or the flavone-3-hydrase gene can be searched for using plant genome information of the target plant based on the nucleotide sequence of the chalcone synthase gene, the chalcone isomerase gene, or the flavone-3-hydrase gene or the amino acid sequence encoded thereby, if the plant genome information has been revealed. As a homologous transcription factor, an amino acid sequence having, for example, 70% or higher, preferably 80% or higher, more preferably 90% or higher, and most preferably 95% or higher homology to the amino acid sequence specifically described above is searched for. Homology values are determined by default using a computer program that implements the BLAST algorithm and a database that stores gene sequence information.

When the plant genome information has not been revealed, the genome is extracted from the target plant, or a cDNA library of the target plant is constructed. The genome region or cDNA hybridizing under stringent conditions to at least part of the nucleotide sequence of the chalcone synthase gene, the chalcone isomerase gene, or the flavone-3-hydrase gene is then isolated. Thus, a homologous gene can be identified. Under stringent conditions, a so-called specific hybrid is formed, but a non-specific

hybrid is not formed. For example, hybridization is carried out at 45°C in the presence of 6× SSC (sodium chloride/sodium citrate), followed by washing at 50°C to 65°C in the presence of 0.2 to 1× SSC and 0.1% SDS. Alternatively, hybridization is carried out at 65°C to 70°C in the presence of 1× SSC, followed by washing at 65°C to 70°C in the presence of 0.3× SSC. Hybridization can be carried out in accordance with a conventional technique, such as the method described in J. Sambrook et al. *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, 1989.

In other words, application of the method for producing plant-derived fat-and-oils of the present invention is not limited to a system involving the use of *Arabidopsis thaliana*-derived seeds, and the method is applicable to any type of plant. Examples of plants to which the method for producing plant-derived fat-and-oils of the present invention can be applied include, but are not limited to, dicotyledonous plants and monocotyledonous plants, such as plants of *Brassicaceae*, *Gramineae*, *Solanaceae*, *Leguminosae*, and *Salicaceae* (see below).

Examples of *Brassicaceae* plants include *Arabidopsis thaliana*, oilseed rape (*Brassica rapa* and *Brassica napus*), cabbage (*Brassica oleracea* var. *capitata*), rapeseed (*Brassica rapa* and *Brassica napus*), field mustard (*Brassica rapa* and *Brassica napus*), *Brassica pekinensis* (*Brassica rapa* var. *pekinensis*), bok choy (*Brassica rapa* var. *chinensis*), turnip (*Brassica rapa* var. *rapa*), *Brassica rapa* var. *hakabura*, *Brassica rapa* var. *lancinifolia*, *Brassica rapa* var. *peruviridis*, pak choy (*Brassica rapa* var. *chinensis*), Japanese radish (*Brassica Raphanus sativus*), and horseradish (*Wasabia japonica*).

Examples of *Solanaceae* plants include tobacco plants (*Nicotiana tabacum*), eggplants (*Solanum melongena*), potatoes (*Solaneum tuberosum*), tomatoes (*Lycopersicon lycopersicum*), capsicum (*Capsicum annuum*), and Petunia plants.

Examples of *Leguminosae* plants include soybeans (*Glycine max*), peas (*Pisum sativum*), horse beans (*Vicia faba*), *Wisteria floribunda*, peanuts (*Arachis hypogaea*), bird's-foot trefoils (*Lotus corniculatus* var. *japonicus*), bush beans (*Phaseolus vulgaris*), azuki beans (*Vigna angularis*), and Acacia plants.

Examples of *Compositae* plants include chrysanthemums (*Chrysanthemum*

morifolium) and sunflowers (*Helianthus annuus*).

Examples of *Arecaceae* plants include *Elaeis guineensis* (or *Elaeis oleifera*), *Cocos nucifera*, *Phoenix dactylifera*, and *Copernicia*.

Examples of *Anacardiaceae* plants include *Rhus succedanea*, *Anacardium occidentale*, *Toxicodendron vernicifluum*, *Mangifera indica*, and pistachios (*Pistacia vera*).

Examples of *Cucurbitaceae* plants include pumpkins (*Cucurbita maxima*, *Cucurbita moschata*, or *Cucurbita pepo*), cucumbers (*Cucumis sativus*), *Trichosanthes cucumeroides*, and gourds (*Lagenaria siceraria* var. *gourda*).

Examples of *Rosaceae* plants include almonds (*Amygdalus communis*), roses (*Rosa*), strawberries (*Fragaria*), cherry trees (*Prunus*), and apples (*Malus pumila* var. *domestica*).

Examples of *Caryophyllaceae* plants include carnations (*Dianthus caryophyllus*).

Examples of *Salicaceae* plants include *Populus nigra* (*Populus trichocarpa*, *Populus nigra*, and *Populus tremula*).

Examples of *Gramineae* plants include maize (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), bamboo (*Phyllostachys*), and sugarcane (*Saccharum officinarum*).

Examples of *Liliaceae* plants include tulips (*Tulipa*) and lilies (*Lilium*).

The phrase "...lack(s) functions of a gene..." refers to a situation in which the relevant gene has been deleted from the genome, in which expression of such gene has been inhibited (at the transcriptional and translational levels), and in which the activity of a protein encoded by such gene has been lowered or eliminated.

Specifically, methods for disrupting a gene of interest are not particularly limited, and examples include a method involving homologous recombination and a method involving the use of transposon. When such gene is to be deleted, the full length of such gene may be deleted, or part thereof may be deleted.

Examples of methods for inhibiting gene expression include, but are not particularly limited to, a method in which a promoter that regulates expression of the

gene of interest is deleted, a method in which a promoter that regulates expression of the gene is substituted with an expression-inducible promoter, a method in which a mutation is introduced into a promoter that regulates expression of the gene, a method in which the gene transcript is degraded with the use of RNA interference, and a method in which translation of the gene is inhibited with the use of antisense RNA.

An example of a method for lowering activity of a protein encoded by the gene of interest is a method in which a substance that specifically binds to a protein of interest to suppress activity thereof is allowed to undergo a reaction. Examples of such substance include an antibody and an inhibitor that can inhibit functions of the protein.

In the method for producing plant-derived fat-and-oils of the present invention, fat-and-oils may be recovered from seeds via, for example, compression, extraction, or expulsion, without particular limitation. For example, fat-and-oil components can be recovered from seeds extracted from plants via ether extraction with the use of a Soxhlet extractor. The method for producing plant-derived fat-and-oils of the present invention involves the use of a plant with higher fat-and-oil content per seed grain, even when the amounts of seeds extracted from individual plants are equivalent. Thus, it can be said that the method of the present invention is excellent in terms of the amount of fat-and-oil production. If the number of individual plants cultivated per unit of cultivation area were constant, in other words, the method for producing plant-derived fat-and-oils of the present invention can significantly improve the fat-and-oil content prepared per unit of cultivation area, which can remarkably decrease the production cost required for fat-and-oil production.

In the method for producing plant-derived fat-and-oils of the present invention, fat-and-oils can be prepared from any plants without particular limitation. Examples include fat-and-oils derived from plants such as soybean oil, sesame oil, olive oil, coconut oil, rice oil, cottonseed oil, sunflower oil, corn oil, safflower oil, and rapeseed oil. In addition, produced fat-and-oils can be extensively utilized for household or industrial applications, and such fat-and-oils can be utilized as starting materials of biodiesel fuels or bioplastics. With the use of the plant of the present invention, specifically, fat-and-oils utilized for household or industrial applications, biodiesel fuels,

bioplastics, or the like can be produced at low cost.

Method for screening for plant exhibiting enhanced fat-and-oil content

According to the present invention, it was discovered that fat-and-oil content was significantly improved in seeds extracted from a strain that lacks a pigment synthesis pathway (reference document: Plant J. Nov. 1995; 8 (5): 659-71), compared with seeds extracted from a wild-type strain. In the pigment synthesis pathway-deficient strain, functions of a gene involved in a pigment synthesis system have been eliminated, and such strain exhibits the phenotype of a lighter seed-coat color (which is closer to white than that of a wild-type seed-coat) compared with a wild-type strain. The tt4 and the Δ CHS strains disclosed in the above document lack the chalcone synthase gene, the tt5 strain lacks the chalcone isomerase gene, and the tt6 strain lacks the flavone-3-hydrase gene. In mutant strains lacking such genes, seed-coat color becomes closer to white because of the failure of pigment synthesis. Thus, seeds may be extracted from plants to be screened for, and seed-coat color of the extracted seeds may be inspected, so that the capacity of the pigment synthesis pathway of the plants for pigment synthesis can be evaluated, and the content thereof in the extracted seeds can be deduced with high accuracy.

When a variety of plants of the same species are present, for example, the seed-coat color of seeds extracted from such plants is observed, and seeds having a color closer to white can be selected as seeds of a plant variety that can produce greater amounts of fat-and-oils. Plants to be screened for may be those obtained via some sort of mutagen treatment or plant varieties prepared via conventional breeding techniques.

Mutagen treatment is not particularly limited, and it may be carried out with the use of a chemical mutagen and/or physical mutagen extensively used for mutagenesis. Examples of chemical mutagens that can be used include ethyl methane sulfonate (EMS), ethylnitrosourea (ENS), 2-aminopurine, 5-bromouracil (5-BU), and an alkylation agent. Examples of physical mutagens that can be used include radioactive rays and ultraviolet rays. Mutagenesis with the use of such mutagens can be carried out by a known method.

According to the screening method of the present invention, the fat-and-oil

content in seeds can be determined in a very simple and rapid manner by visually observing seed-coat color without the need to destroy seeds extracted from plants.

According to the screening method of the present invention, also, the seed-coat color of seeds extracted from plants may be determined based on image data and quantitatively assayed. More specifically, the image of the seed to be evaluated is converted into digital data, and the R, G, and B values (i.e., the RGB value) in the seed region in the image data are assayed. The R, G, and B values in the seed region may be assayed with the use of any type of image-processing software. Subsequently, the assayed R, G, and B values are compared with the R, G, and B values in seeds extracted from a wild-type plant. For example, the integrated value of the assayed R, G, and B values is determined and compared with that of seeds extracted from a wild-type plant. If the integrated value of the assayed R, G, and B values is significantly higher than that of seeds extracted from a wild-type plant, for example, the seed-coat color of the seed to be evaluated can be determined to be closer to white. When the integrated value of the assayed R, G, and B values is 2.88 or more times as high as that of seeds extracted from a wild-type plant, in particular, the seed can be determined to have a color closer to white (i.e., to be more pale in color).

As described above, the fat-and-oil content in seeds can be determined in a very simple and rapid manner without the need for destroying seeds, even with a technique of observing the seed-coat color of a seed extracted from a plant as image data. When observing the seed-coat color of a seed in image data, the sum of the R, G, and B values or the like may be calculated, in addition to the integrated value of the R, G, and B values.

Examples

Hereafter, the present invention is described in greater detail with reference to the examples, although the technical scope of the present invention is not limited to the examples.

[Example 1]

In this example, chimeric proteins (i.e., fusion proteins) of the *Arabidopsis*

thaliana transcription coactivator At5g24520 and transcription factor At1g71030 to which repressor domain sequences had been added were expressed in plants, and the fat-and-oil content in seeds obtained from the plants was measured. For comparison, a chimeric protein (i.e., a fusion protein) of the transcription factor At1g56650 was expressed in a plant in the same manner, and the fat-and-oil content in seeds was assayed.

Amplification of transcription factor gene

A DNA fragment of a coding region of At1g71030 excluding the termination codon thereof, a DNA fragment of a coding region of At1g71030 including the termination codon thereof, a DNA fragment of a coding region of At5g24520 excluding the termination codon thereof, and a DNA fragment of a coding region of At1g56650 excluding the termination codon thereof were obtained from the *Arabidopsis thaliana* cDNA library, and the DNA fragments were amplified via PCR using the primers shown below. PCR was carried out via denaturation at 94°C for 1 minute, annealing at 47°C for 2 minutes, and elongation at 74°C for 1 minute, and this cycle was repeated 25 times. After the completion of PCR, the amplified DNA fragments were separated via agarose gel electrophoresis and recovered.

Forward primer 1 for amplifying At1g71030

gATGAACAAAACCCGCCTTCGTGCTCTCTC (SEQID NO: 11)

Reverse primer 1 for amplifying At1g71030

TCGGAATAGAAGAAGCGTTTCTTGACCTGT (SEQID NO: 12)

Forward primer 2 for amplifying At1g71030

gATGAACAAAACCCGCCTTCGTGCTCTCTC (SEQID NO: 13)

Reverse primer 2 for amplifying At1g71030 (SEQID NO: 14)

TCATCGGAATAGAAGAAGCGTTTCTTGACC

Forward primer for amplifying At1g56650

GATGGAGGGTTCGTCCAAAGGGC (SEQID NO: 15)

Reverse primer for amplifying At1g56650

ATCAAATTTACAGTCTCTCCATCG (SEQID NO: 16)

Forward primer for amplifying At5g24520

gATGGATAATTCAGCTCCAGATTCGTTATC (SEQID NO: 17)

Reverse primer for amplifying At5g24520

AACTCTAAGGAGCTGCATTTTGTAGCAAA (SEQID NO: 18)

Preparation of fusion gene

In order to add the repressor domain sequence to the 3' terminus of the transcription factor gene encoded by the above DNA fragment, a p35SSXG vector having the *Sma*I site and the repressor domain (amino acid sequence: GLDLLELRGFA) sequence downstream of the CaMV35S promoter was used. In order to ligate the transcription factor gene sequence to the repressor domain sequence, the vector was cleaved with *Sma*I, and a PCR-amplified fragment encoding the above transcription factor was introduced to prepare p35SSXG (At1g56650), p35SSXG (At5g24520), and p35SSXG (At1g71030). The PCR-amplified fragment obtained with the use of the forward primer 1 for amplifying At1g71030 and the reverse primer 1 for amplifying At1g71030 was introduced into p35SSXG (At1g71030). In order to express the PCR-amplified fragment obtained with the use of the forward primer 2 for amplifying At1g71030 and the reverse primer 2 for amplifying At1g71030 without the addition of the repressor domain, a p35SOXG vector having the *Sma*I site sequence downstream of the CaMV35S promoter was introduced into the *Sma*I-cleavage site to prepare p35SOXG (At1g71030).

Construction of modified transcription factor and transcription factor expression vector

A pBCKH binary vector was used in order to introduce a gene into a plant by the *Agrobacterium* method. This vector was prepared by incorporating a cassette of the Gateway vector conversion system (Invitrogen) into the *Hind*III site of pBIG (Hygr) (Nucleic Acids Res. 18, 203, 1990). In order to incorporate the modified transcription factor gene into this vector, the vector was mixed with p35SSXG (At1g56650), p35SSXG (At5g24520), p35SSXG (At1g71030), or p35SOXG (At1g71030), and a recombination reaction was carried out using GATEWAY^{*}LR clonase (Invitrogen). As a result, pBCKH-p35SSXG (At1g56650), pBCKH-p35SSXG (At5g24520), pBCKH-p35SSXG (At1g71030), and pBCKH-p35SOXG (At1g71030) were constructed.

Introduction of expression vector for modified transcription factor gene into plant

* Trade-mark

Arabidopsis thaliana, Columbia was used as a plant into which the modified transcription factor was to be introduced. Gene introduction was carried out in accordance with the vacuum infiltration method of transformation of *Arabidopsis thaliana*. Plants were infected by being soaked in the *Agrobacterium* solution without depressurization. Specifically, expression vectors for modified transcription factors, pBCKH-p35SSXG (At1g56650), pBCKH-p35SSXG (At5g24520), pBCKH-p35SSXG (At1g71030), and pBCKH-p35SOXG (At1g71030), were introduced into soil bacteria (i.e., the *Agrobacterium tumefaciens* strain GV3101 (CS8CIRifr) pMP90 (Gmr)) (Koncz and Schell, 1986) via electroporation.

The introduced bacteria were cultured in 1 liter of YEP medium containing antibiotics (50 µg/ml of kanamycin (Km), 25 µg/ml of gentamicin (Gm), and 50 µg/ml of rifampicin (Rif)) until OD600 reached 1. Subsequently, the bacteria were recovered from the culture solution and suspended in 1 liter of infiltration medium (containing 2.2 g of MS salt, 1x B5 vitamin, 50 g of sucrose, 0.5 g of MES, 0.044 µM of benzylaminopurine, and 400 µl of Silwet per liter; pH: 5.7). The *Arabidopsis thaliana* plant that had been grown for 14 days was soaked in this solution for 1 minute, the plant was infected, and culture was continued again for fructification. The resulting seeds (T1 seeds) were sterilized with a 50% bleach/0.02% Triton X-100* solution for 7 minutes, the seeds were rinsed three times with sterilized water, and the seeds were sowed on the sterilized hygromycin selection medium (4.3 g/l MS salt, 0.5 % sucrose, 0.5 g/l MES (pH 5.7), 0.8% agar, 30 mg/l hygromycin, and 250 mg/l vancomycin). Ten transformed strains that had grown on the hygromycin plate (T1 plants) were selected per modified transcription gene and transferred to a pot (diameter: 50 mm) containing vermiculite composite soil. The transformants were cultivated at 22°C for 16 hours in the light and 8 hours in the dark at an optical intensity of about 60 to 80 µE/cm² to obtain seeds (T2 seeds). The epidermis color of the obtained T2 seeds was light brown or yellow while that of the wild-type strain was dark brown.

Strain that lacks pigment synthesis pathway

In this example, the fat-and-oil content in seeds obtained from strains that lack the pigment synthesis pathway was measured. Specifically, seeds were obtained from

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strains that lack the pigment synthesis pathway; i.e., the *tt4* strain (NASC stock No. N85) (reference: Plant J., 8, 659-671, 1995), the *tt5* strain (NASC stock No. N86), the *tt6* strain (NASC stock No. N87) (reference: Plant Physiol., 111, 339-345, 1996), and the Δ CHS strain (NASC stock No. N520583) (NASC: the Nottingham Arabidopsis Stock Centre). The *tt4*, *tt5*, and *tt6* strains were prepared from the *Arabidopsis thaliana*, Ler strain, and the Δ CHS strain was prepared from the *Arabidopsis thaliana* Col-0 strain. The resulting seeds were sterilized with a 50% bleach/0.02% Triton X-100 solution for 7 minutes, rinsed three times with sterilized water, and sowed on a medium (4.3 g/l MS salt, 0.5 % sucrose, 0.5 g/l MES (pH 5.7), 0.8% agar). The plants growing on the plate were transferred to a pot (diameter: 50 mm) containing vermiculite composite soil. The *tt4*, *tt5*, *tt6*, and WT (Ler) strains were cultivated at 22°C for 16 hours in the light and 8 hours in the dark at an optical intensity of about 50 to 60 $\mu\text{E}/\text{cm}^2$, or the Δ CHS and WT (Col-0) strains were cultivated at an optical intensity of about 40 $\mu\text{E}/\text{cm}^2$ to obtain seeds. The epidermis color of the obtained seeds was light brown or yellow while that of the wild-type strain was dark brown.

Analysis of T2 seeds into which modified transcription factor or transcription factor had been introduced

The fat-and-oil content of the T2 seeds into which either of the modified transcription factor gene or improved transcription coactivator gene had been introduced (i.e., (At1g56650-SRDX, At5g24520-SRDX, or At1g71030-SRDX), the T2 seeds into which a transcription factor had been introduced (i.e., At1g71030), and the wild-type seeds (Col-0 and Ler strains) were analyzed. Quantitative analysis of the fat-and-oil content in 2 to 10 mg of *Arabidopsis thaliana* seeds was carried out using MARAN-23 (Resonance Instruments Ltd., UK)^H-NMR and the RI-NMR Ver. 2.0 analysis software. A calibration curve was prepared using olive oil as a fat-and-oil reference material, and the fat-and-oil content in seeds (% by weight) was determined.

Average values for the fat-and-oil content in seeds of strains into which the modified transcription factor genes, the improved transcription coactivators, or the transcription factor genes had been introduced and wild-type strains were determined ($n = 3$ to 10). As a result, the percentages of fat-and-oil content increase when the

average fat-and-oil content of Col-0 was designated as 1 were as follows: 30.2% in the T2 seeds (At1g56650-SRDX), 12.3% in the T2 seeds (At5g24520-SRDX), 12.2% in the T2 seeds (At1g71030-SRDX), and 2.3% in the T2 seeds (At1g71030) (Fig. 1)

Analysis of seeds of strain that lacks pigment synthesis pathway

The fat-and-oil content in four types of seeds of strains that lack the pigment synthesis pathway (i.e., tt4, tt5, tt6, and Δ CHS strains) and of wild-type strains (Col-0 and Ler strains) were analyzed. Quantitative analysis of the fat-and-oil content in 2 to 10 mg of *Arabidopsis thaliana* seeds was carried out using MARAN-23 (Resonance Instruments Ltd., UK)^H-NMR and the RI-NMR Ver. 2.0 analysis software. A calibration curve was prepared using olive oil as a fat-and-oil reference material and the fat-and-oil content in seeds (% by weight) was determined.

Average values of the fat-and-oil content in seeds of strains that lack a pigment synthesis pathway were determined (n = 3 to 10). The fat-and-oil content of the Δ CHS strain was 8.9% relative to that of the Col-0 strains, and the fat-and-oil content of the tt4, tt5, and tt6 strains were 4.7%, 8.8%, and 11.1%, respectively, relative to that of the Ler strain (Fig. 2).

Results and discussion

The above results demonstrate that the fat-and-oil content per seed weight of plants into which the chimeric genes of the transcription factor At1g56650, the transcription coactivator At5g24520, and the transcription factor At1g71030 each comprising the repressor domain had been introduced were higher than that per weight of wild-type strains that had been simultaneously cultivated, and such plants are very effective for fat-and-oil production. Fat-and-oil content per seed weight of the plant into which At1g71030 having expression accelerating activity had been introduced was somewhat increased compared with the fat-and-oil content per weight of the plant that had been simultaneously cultivated. However, the percentage of increase was about one-fifth the percentage of increase of the fat-and-oil content per weight of a plant seed into which At1g71030 with suppressed expression accelerating activity had been introduced. At1g71030 encodes a protein having a single MYB-like domain (AtMybL2) and overexpresses this gene with the aid of the CaMV35S promoter to show

the traits of a lack of trichomes on leaves, stem, and calyx. This is considered to result from suppression of expression of the GL2 gene, which is necessary for trichome formation (reference: DNA Res., 9, 31-34, 2002). It is reported that disruption of the GL2 gene increases the fat-and-oil content in seeds by 8% (reference: Plant Mol Biol., 2006, 60, 377-87, 2006).

Also, the AtMybL2 protein has a transcription repressor comprising 6 amino acids at the carboxy terminus, and synthesis of an anthocyanin precursor was suppressed in a plant overexpressing the AtMybL2 gene and a plant overexpressing the gene encoding AtMybL2 to which a transcription repressor known as the EAR-motif had been added (reference: 18TH International Conference On Arabidopsis Research, TAIR accession Publication: 501721814). As a result of analysis, the percentage of increase in the fat-and-oil content in the T2 seeds obtained from At1g71030-overexpressing plants was 2.3%. The percentage of increase in the fat-and-oil content of the T2 seeds obtained from the At1g71030-overexpressing plant to which the repressor domain had been added was significantly high (i.e., 12.2%), which was remarkably high compared with the percentage of increase in the fat-and-oil content when the GL2 gene was disrupted (i.e., 8%). Based on these results, it is considered that the repressor domain-containing At1g71030 functions during the process of seed fat-and-oil synthesis and storage in unknown pathways other than GL2 and increases fat-and-oil content.

As a result of analysis of strains lacking the pigment synthesis pathway, the fat-and-oil content in seeds of the mutant strains tt4, tt5 and tt6, in which the major gene of the pigment synthesis pathway had been disrupted, and in seed of the ΔCHS strain in which the CHS gene had been disrupted upon insertion of T-DNA were higher than that in wild-type strains. Regarding the correlation between seed-coat color and fat-and-oil content, it is reported that the fat-and-oil content of the yellow-seeded rapeseed cultivar, HUA-yellow No. 1, is 5% to 7% higher than that of the black-seeded rapeseed cultivar (reference: Genome 44: 1077-1082, 2001). According to a conventional breeding technique resulting from interbreeding, however, a similar phenomenon can be observed even when the loci of traits that determine the seed-coat color and the fat-and-oil content in seeds are adjacent to each other. Thus, the correlation between gene expression and

traits has not yet been elucidated. Specifically, there was no finding regarding the influence of the gene locus that affects the trait of seed-coat color on fat-and-oil content in the past.

In the present invention, however, the gene encoding a pigment synthesis enzyme of a seed coat was actually disrupted, and an increase was observed in the fat-and-oil content in seeds. Thus, it was found for the first time that the seed-coat color is a critical phenotype for predicting the fat-and-oil content in the case of gene introduction or molecular breeding via gene disruption, in addition to conventional breeding via crossing. The use of a seed-coat color as an indicator enables efficient selection of seeds exhibiting increased fat-and-oil content in a non-destructive manner without the use of special equipment.

More specifically, seeds of wild-type strains and At1g71030-SRDX, At1g56650-SRDX, and Δ CHS strains were photographed and the images were converted into digital data. Image processing software (Adobe Photoshop) was applied to the resulting digital data to quantify the RGB values in the seed regions. Subsequently, the integrated value of the quantified R, G, and B values was determined. In addition, the ratio relative to the integrated value of the quantified R, G, and B values of wild-type strains was determined. The results are shown in Table 1 and in Fig. 3.

Table 1

Photography lot	Strain	Average			Sample standard deviation			RxGxB	Increase in RxGxB value relative to WT
		R	G	B	R	G	B		
1	WT (Col-o)	129.7	82.9	48.9	13.5	13.7	16.1	525,060	1.00
	atlg71030-SRDX	170.1	132.8	66.9	13.2	17.5	22.8	1,510,321	2.88
	atlg56650-SRDX	172.1	133.9	69.2	13.0	13.3	19.0	1,594,257	3.04
	ΔCHS	177.8	139.9	64.2	16.1	16.2	18.0	1,595,139	3.04
2	WT (Col-o)	159.1	84.8	59.8	14.3	12.1	14.7	806,880	1.00
	atlg56650-SRDX	197.9	182.4	126.5	12.3	12.7	13.2	4,564,621	5.66
3	WT (Col-o)	154.9	96.8	40.1	48.2	37.3	16.0	600,644	1.00
	atlg56650-SRDX	166.4	156.1	104.6	28.3	33.0	51.4	2,717,975	4.53

As shown in Table 1 and Fig. 3, the integrated value of the R, G, and B values of the At1g71030-SRDX strain, the At1g56650-SRDX strain, and the Δ CHS strain was at least 2.88 times higher than that of wild-type strains. Thus, seed-coat color can be quantitatively assayed with the use of image data of seeds, and the fat-and-oil content in seeds can be evaluated in a very simple and rapid manner.

CLAIMS:

1. A plant cell in which a chimeric protein resulting from the fusion of a transcription factor with a functional peptide,

wherein the transcription factor is:

- (a) a protein comprising the amino acid sequence as shown in SEQ ID NO: 4; or
- (b) a protein encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence as shown in SEQ ID NO: 3 and having transcription accelerating activity, wherein the stringent conditions comprise hybridization at 45 °C in the presence of 6X sodium chloride/sodium citrate (SCC) and washing at 50 °C to 60 °C in the presence of 0.2X to 1X SCC and 0.1% sodium dodecyl sulfate (SDS);

and wherein the functional peptide comprises the amino acid sequence represented by any of formulae (1) to (8) below:

- (1) X1-Leu-Asp-Leu-X2-Leu-X3, wherein X1 represents 0 to 10 amino acid residues; X2 represents Asn or Glu; and X3 represents at least 6 amino acid residues;
- (2) Y1-Phe-Asp-Leu-Asn-Y2-Y3, wherein Y1 represents 0 to 10 amino acid residues; Y2 represents Phe or Ile; and Y3 represents at least 6 amino acid residues;
- (3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3, wherein Z1 represents Leu, Asp-Leu, or Leu-Asp-Leu; Z2 represents Glu, Gln, or Asp; and Z3 represents 0 to 10 amino acid residues;
- (4) Asp-Leu-Z4-Leu-Arg-Leu, wherein Z4 represents Glu, Gln, or Asp;
- (5) α 1-Leu- β 1-Leu- γ 1-Leu;
- (6) α 1-Leu- β 1-Leu- γ 2-Leu;
- (7) α 1-Leu- β 2-Leu-Arg-Leu; or
- (8) α 2-Leu- β 1-Leu-Arg-Leu

wherein, in formulae (5) to (8), α 1 represents Asp, Asn, Glu, Gln, Thr, or Ser; α 2 represents Asn, Glu, Gln, Thr, or Ser; β 1 represents Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His; β 2 represents Asn, Arg, Thr, Ser, or His; γ 1 represents Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp; and γ 2 represents Gln, Asn, Thr, Ser, His, Lys, or Asp.

2. The plant cell according to claim 1, wherein transcription accelerating activity of the transcription factor is suppressed.
3. The plant cell according to claim 1, wherein the chimeric protein has transcription repressor activity.
4. The plant cell according to any one of claims 1 to 3, wherein the fat-and-oil content in a given tissue comprising the plant cell is greater than the fat-and-oil content in corresponding plant tissue comprising a plant cell lacking the chimeric protein.
5. A method for producing fat-and-oil using a plant comprising a step of separating and recovering fat-and-oil from a plant derived from the plant cell according to any one of claims 1 to 4.
6. A method for producing fat-and-oil using a plant comprising a step of separating and recovering fat-and-oil from plant tissue comprising plant cells according to any one of claims 1 to 4.

Fig. 1

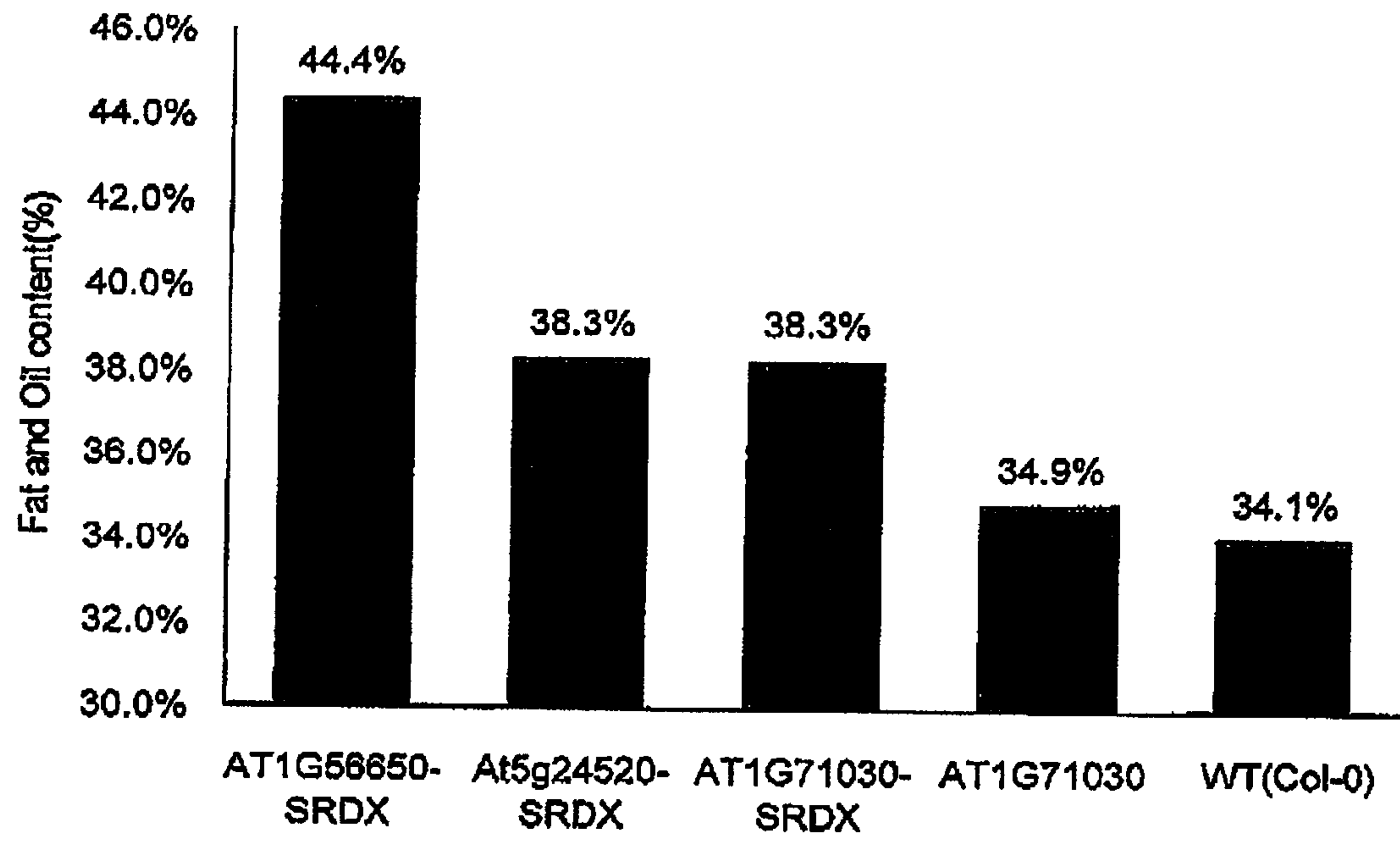


Fig. 2

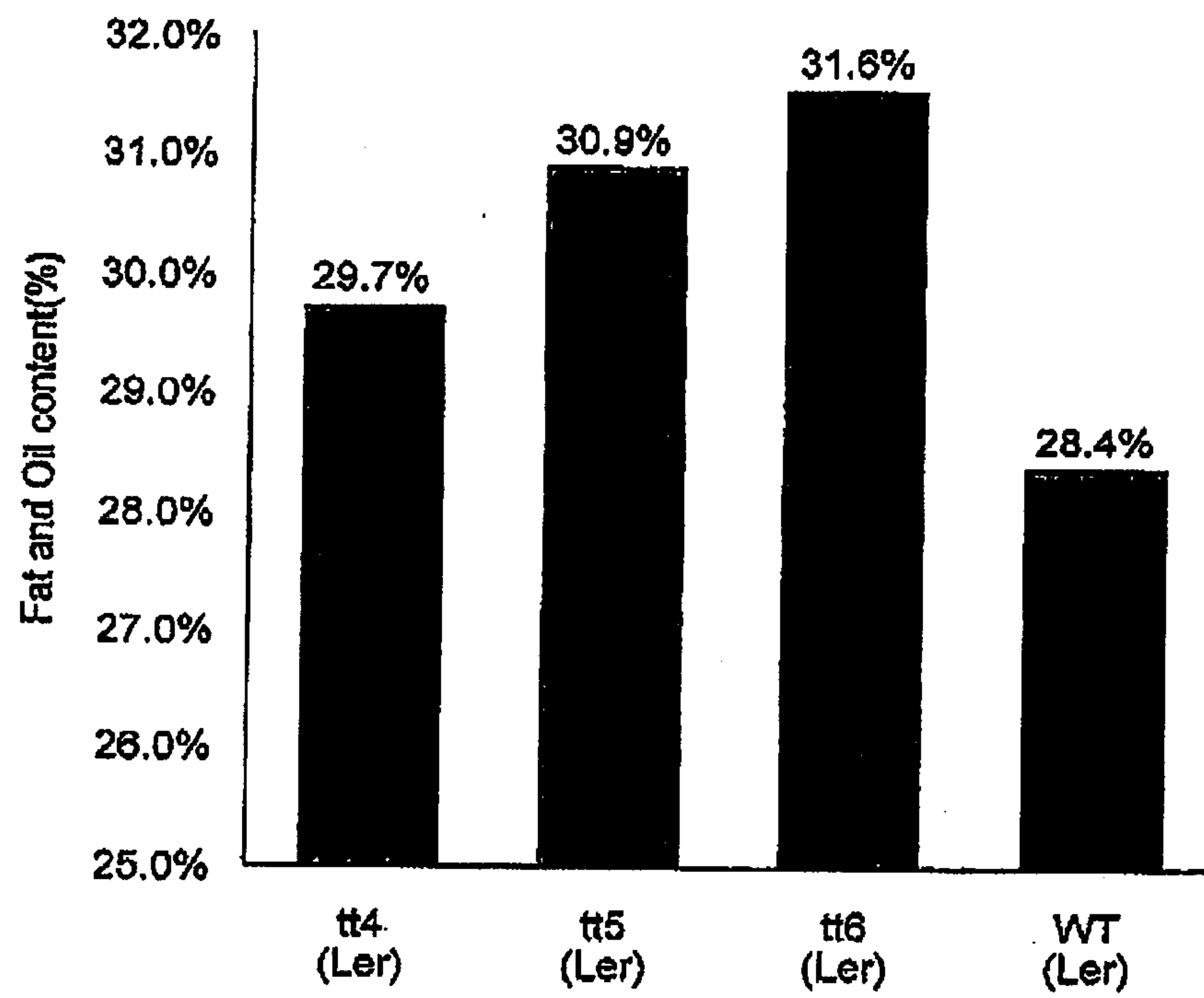


Fig. 3

