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(54) **Title**: GENE CAPABLE OF INCREASING QUANTITY OF BIOMASS AND/OR SEED OF PLANT, AND USE THEREOF

(54) 発明の名称 : 植物のバイオマス量及び／又は種子量を増産させる遺伝子及びその利用方法

[図8]

AA TRANSGENIC PLANT (REFERENCE EXAMPLE 1)
BB WILD TYPE (ARABIDOPSIS)
CC TRANSGENIC PLANT (EXAMPLE 1)

(57) **Abstract**: Disclosed is a technique for increasing the quantity of a plant biomass to a great extent. The technique involves introducing both a gene which encodes protein phosphatase 2C that comprises three consensus sequences respectively comprising the amino acid sequences depicted in SEQ ID NO:1 to SEQ ID NO:3 lying from the N-terminal in this order and a gene which encodes glutathione-bound plastid-type fructose-1,6-bisphosphate aldolase, or modifying an endogenous expression regulation region for the gene.

(57) 要約: 植物バイオマス量を大幅に増産できる技術を提供する。 配列番号１～３に示すアミノ酸配列からなる３つの共通配列をＮ末端側からこの順で有するプロテインホスファターゼ 2C をコードする遺伝子及びグルタチオン結合性プラスチド型フルクトース 1,6-ビスリン酸アルドラーゼをコードする遺伝子を導入する、又は内在する当該遺伝子の発現制御領域を改変する。

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DESCRIPTION

GENE FOR INCREASING THE PRODUCTION OF PLANT BIOMASS AND/OR SEEDS AND METHOD FOR USE THEREOF

Technical Field

[0001]

The present invention relates to: a plant into which a given gene is introduced or an expression control region of an endogenous gene corresponding to the given gene is modified; a method for increasing the production of biomass and/or seeds through introduction of a given gene or modification of an expression control region of an endogenous gene corresponding to the given gene; and a method for producing a plant capable of producing an increased amount of biomass and/or seeds.

Background Art

[0002]

The term "biomass" generally refers to the total amount of organisms that inhabit or exist in a given area. When such term is used with regard to plants, in particular, it refers to dry weight per unit area. Biomass units are quantified in terms of mass or energy. The expression "biomass" is synonymous with "Seibutsutairyo" or "Seibutsuryo." In the case of plant biomass, the term "standing crop" is occasionally used for "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 of plant biomass is effective for global environmental preservation, the prevention of global warming, and mitigation of greenhouse gas emissions. Thus, technologies for increasing the production of plant biomass have been industrially significant.

[0003]

Plants are cultivated for the purpose of using some tissues thereof (e.g., seeds, roots,

leaves, or stems) or for the purpose of producing various materials, such as fats and oils. Examples of fats 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 fats and oils are extensively used for household and industrial applications. Also, fats and oils produced from plants are used as raw materials for biodiesel fuel or bioplastic, and the applicability thereof is increasing for alternative energy to petroleum.

[0004]

In particular, an energy crop such as sugar cane can be used as a raw material for biofuel. Hence, the increased production of the total mass of a plant itself (the amount of plant biomass) is expected. Under such circumstances, improvement in productivity per unit of cultivation area is required in order to increase the production of the amount of plant biomass. It has been found that if the number of cultivated plants is assumed to be constant per unit of cultivation area, improvement in the amount of biomass per plant would be necessary.

[0005]

However, it is thought that since many genes are involved in the amount of plant biomass (a so-called "kind of quantitative trait"), individual gene introduction or individual genetic modification cannot lead to an effective increase in production. Meanwhile, a great deal of difficulties are associated with introduction of many genes in a desired state into a plant. Such gene introduction is also problematic in that if successful introduction takes place, desirable traits cannot always be acquired.

[0006]

Various gene introduction techniques are known as techniques for increasing the production of plant biomass, as disclosed in Patent Documents 1-7, for example. However, none of these techniques can be said to exhibit sufficient effects of increasing the production of biomass.

[0007]

Also, patent document 8 discloses a transformed plant with improved growth potential and disease resistance through overexpression of a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene (FBA1 gene).

[Prior Art Documents]

[Patent Documents]

[0008]

[Patent Document 1] JP Patent Publication (Kohyo) No. 2001-505410 A

[Patent Document 2] JP Patent Publication (Kohyo) No. 2001-519659 A

[Patent Document 3] JP Patent Publication (Kohyo) No. 2007-530063 A

[Patent Document 4] JP Patent Publication (Kokai) No. 2005-130770 A

[Patent Document 5] JP Patent Publication (Kohyo) No. 2000-515020 A

[Patent Document 6] JP Patent Publication (Kohyo) No. 9-503389 A (1997)

[Patent Document 7] JP Patent Publication (Kokai) No. 2005-52114 A

[Patent Document 8] WO2007/091634

[0008A]

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0008B]

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

[Summary of the Invention]

[0009]

In view of the above circumstances, the inventors searched for genes having novel functions of drastically improving the amount of plant biomass and thus attempted to provide a technique with which the production of plant biomass can be drastically increased.

[0010]

As a result of intensive studies, the present inventors have made the novel finding that the production of plant biomass can be drastically increased by introducing a gene encoding protein phosphatase 2C having characteristic consensus

sequences and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase, or modifying an expression control region of endogenous genes corresponding to the genes. Thus, they have completed the present invention.

[0011]

Specifically, the plant according to the present invention is a plant in which a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase are introduced, or an expression control region of endogenous genes corresponding to the genes is modified.

[0012]

Also, the method for increasing the production of biomass according to the present invention comprises introducing a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase, or modifying an expression control region of endogenous genes corresponding to the genes.

[0013]

Furthermore, the method for producing a plant according to the present invention comprises the steps of: preparing a transformed plant in which a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase are introduced, or an expression control region of endogenous genes corresponding to the genes is modified; and measuring the amount of biomass of a progeny plant of the transformed plant and then selecting a line in which the amount of biomass is significantly improved.

[0014]

In the present invention, the above gene encoding protein phosphatase 2C can be at

least one type of gene selected from the group consisting of At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270, or a gene functionally equivalent thereto.

[0015]

In the present invention, the gene encoding protein phosphatase 2C preferably encodes any one of the following proteins (a) to (c):

[0016]

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 5;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5 and having protein phosphatase 2C activity; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 4 and has protein phosphatase 2C activity.

Also, in the present invention, an example of the above functionally equivalent gene is a protein phosphatase 2C gene from an organism other than *Arabidopsis thaliana.* Another example of an organism other than *Arabidopsis thaliana* is one type of organism selected from the group consisting of rice (*Oryza sativa*), black cottonwood (*Populus trichocarpa*), European grape (*Vitis vinifera*), *Medicago truncatula* (*Medicago truncatula*), alfalfa (*Medicago sativa*), *Physcomitrella patens* (*Physcomitrella patens*), ice plant (*Mesembryanthemum crystallinum*), *Chlamydomonas reinhardtii* (*Chlamydomonas reinhardtii*), corn (*Zea mays*), rapeseed (*Brassica rapa*), tomato (*Solanum lycopersicum*), monkey flower (*Mimulus guttatus*), and monocellular red alga (*Cyanidioschyzon merolae*).

[0017]

In the present invention, the above gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase can be an At2g01140 gene or a gene functionally

equivalent to the gene.

[0018]

In the present invention, the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase preferably encodes any one of the following proteins (a) to (c):

[0019]

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 32;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 32 and exhibiting fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 31 and exhibits fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione.

Also, in the present invention, an example of the above functionally equivalent gene is a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene from an organism other than *Arabidopsis thaliana*. Another example of an organism other than *Arabidopsis thaliana* is one type of organism selected from the group consisting of rice (*Oryza sativa*), European grape (*Vitis vinifera*), castor-oil plant (*Ricinus communis*), black cottonwood (*Populus trichocarpa*), sitka spruce (*Picea sitchensis*), and corn (*Zea mays*).

[0020]

Examples of plants to be subjected to the present invention include dicotyledons such as plants of the family Brassicaceae. Examples of plants of the family Brassicaceae include *Arabidopsis thaliana* and rapeseed. Other examples of plants to be subjected to the present invention include monocotyledons such as plants of the family Gramineae. Examples of plants of the family Gramineae include rice and sugarcane.

[0021]

This description hereby incorporates the entire content of the description and/or the drawings of Japanese Patent Application No. 2009-060154, which is the basis of the priority claim of this application.

[Effect of the Invention]

[0022]

The plant according to the present invention is a plant capable of producing significantly improved amount of biomass and/or seeds compared with wild-type plants. Also, the method for increasing the production of biomass and/or seeds according to the present invention can realize the significantly increased production of biomass and/or seeds compared with target wild-type plants. Furthermore, the method for producing a plant according to the present invention makes it possible to produce a plant capable of producing significantly improved amount of biomass and/or seeds compared with wild-type plants. Therefore, through application of the present invention, for example, productivity can be improved when the plant itself is a product and this can be achieved at lower cost. Also, through application of the present invention, for example, the productivity can be improved when seeds are directly products or ingredients contained in seeds are directly products and this can be achieved at lower cost.

[Brief Description of the Drawings]

[0023]

Fig. 1-1 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270.

Fig. 1-2 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250,

At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270.

Fig. 1-3 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270.

Fig. 2-1 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7.

Fig. 2-2 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7.

Fig. 2-3 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7.

Fig. 3-1 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by EEF02079, ABK94899, EEE88847, EEF36097, CAO42215, At2g01140, ABK24286, ABK25226, ABK24568, ACG47464, ACG47669, and Os01g0118000.

Fig. 3-2 is a characteristic diagram showing the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program for amino acid sequences encoded by EEF02079, ABK94899, EEE88847, EEF36097, CAO42215, At2g01140, ABK24286, ABK25226, ABK24568, ACG47464, ACG47669, and Os01g0118000.

Fig. 4 is a phylogenetic tree created for EEF02079, ABK94899, EEE88847, EEF36097, CAO42215, At2g01140, ABK24286, ABK25226, ABK24568, ACG47464, ACG47669, Os01g0118000, At4g38970, At2g21330, and BAA77604.

Fig. 5 is a photo showing the above-ground parts of wild-type plants and transformed plants into which a fragment containing ORF of PP2C (protein phosphatase 2C) gene (At3g05640) was introduced.

Fig. 6 is a characteristic diagram showing the results of measuring the amounts of biomass of the above-ground parts of wild-type plants and transformed plants into which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) was introduced. The result for the wild-type plants is the average value for 12 individual wild-type plants and each result for the transformed plants is the average value for 5 individual transformed plants.

Fig. 7 is a characteristic diagram showing the results of measuring the amounts of seeds of wild-type plants and transformed plants into which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) was introduced. The result for the wild-type plants is the average value for 12 individual wild-type plants and each result for the transformed plants is the average value for 5 individual transformed plants.

Fig. 8 is a photo showing the above-ground parts of wild-type plants, transformed plants produced by introducing a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640), and transformed plants produced by introducing an FBA1 gene (At2g01140) into the above-mentioned transformed plants (into which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) had been introduced).

Fig. 9 is a characteristic diagram showing the results of measuring the amounts of biomass of the above-ground parts of transformed plants produced by introducing a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640), as well as transformed plants produced by introducing an FBA1 gene (At2g01140) into the aforementioned transformed plants (into which a fragment containing ORF of the PP2C

(protein phosphatase 2C) gene (At3g05640) had been introduced).

[Best Mode for Carrying Out the Invention]

[0024]

The present invention will be described in detail as follows.

[0025]

The plant according to the present invention is produced by introducing a gene encoding protein phosphatase 2C having characteristic consensus sequences and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase or modifying an expression control region of endogenous genes corresponding to the genes, wherein the amount of biomass is significantly improved compared with wild-type plants. The expression level of a target gene can be significantly increased compared with that in a wild-type plant by exogenously introducing the target gene into plants or modifying an expression control region of an endogenous gene corresponding to the gene. The plant according to the present invention may be produced by causing the expression of the protein phosphatase 2C gene and the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene in all plant tissues, or at least in some plant tissues. Here, the term "plant tissue(s)" is meant to include plant organ(s) such as leaves, stems, seeds, roots, and flowers.

[0026]

Also, the term "expression control region" refers to a promoter region to which RNA polymerase binds and a region to which another transcription factor binds. A transcriptional regulatory region is preferably modified by substituting a promoter region, for example, among endogenous transcriptional regulatory regions with a promoter region that enables a higher expression level.

[0027]

Protein phosphatase 2C gene

In the present invention, the protein phosphatase 2C gene encodes protein phosphatase 2C that has 3 consensus sequences comprising the amino acid sequences shown

in SEQ ID NOS: 1-3 from the N-terminal side in such order. In addition, a gene group classified as Group E as in Figure 1 of Topographic cladogram (on page 237 of Reference: TRENDS in Plant Science Vol. 9 No. 5 May 2004 pages 236-243) encodes protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order. In addition, the reference predicts the presence of 76 protein phosphatase 2C genes in *Arabidopsis thaliana* and discloses the results of producing a phylogenetic tree of these genes using T-Coffee software (reference; Notredame, C. et al. 2000 T-Coffee: a novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 302, 205-247) as in Figure 1. In this phylogenetic tree, protein phosphatase 2C genes classified as members of Group E encode protein phosphatase 2C that has 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order. The 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 are characteristic sequences in Group E in the above-mentioned classification and serve as a basis for clear differentiation from other groups.

[0028]

Group E in the above classification includes protein phosphatase 2C genes specified by *Arabidopsis thaliana*-derived At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270. Fig. 1 shows the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program (which can be used with the DDBJ of the National Institute of Genetics (http://clustalw.ddbj.nig.ac.jp/top-j.html)) for the amino acid sequences encoded by these *Arabidopsis thaliana*-derived protein phosphatase 2C genes, At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050 and At3g06270 (with the amino acid (sequence) substitution matrix used herein being a default matrix known as BLOSUM

(Blocks of Amino Acid Substitution Matrix)). As shown in Fig. 1, these protein phosphatase 2C genes classified as members of Group E have consensus sequences characteristic in the regions denoted as I to III. These regions denoted as I to III are subjected with a rice-derived protein phosphatase 2C gene (described later) to alignment analysis, so that the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 can be defined.

[0029]

Herein, in the amino acid sequence shown in SEQ ID NO: 1, which is an amino acid residue denoted as "Xaa," may be any amino acid, and it is not limited to any particular amino acid. However, the 1st amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 1 is preferably leucine (three character code: Leu and single character code: L; the same applies to the following) or phenylalanine (Phe, F). The 4th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 1 is preferably valine (Val, V), isoleucine (Ile, I), or methionine (Met, M). The 16th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 1 is preferably serine (Ser, S) or alanine (Ala, A). The 17th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 1 is preferably lysine (Lys, K), arginine (Arg, R), glutamine (Gln, Q), or asparagine (Asn, N). More specifically, a consensus sequence comprising the amino acid sequence shown in SEQ ID NO: 1 is preferably (L/F)XG(V/I/M)FDGHGXXGXXX(S/A)(K/R/Q/N)XV. In such amino acid sequence, pluralities of amino acids in parentheses represent possible variations of amino acid residues at the relevant positions. Also, in the following amino acid sequences, "X" means that any amino acid residue may be present at the relevant position.

[0030]

Also, such a consensus sequence may be a sequence containing the following 3 amino acid residues on the N-terminal side of Region I in Fig. 1: (D/E/N)XX.

[0031]

Here, in the amino acid sequence shown in SEQ ID NO: 2, an amino acid residue denoted as "Xaa," may be any amino acid, and it is not limited to any particular amino acid. However, the 5th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably glycine (Gly, G), alanine (Ala, A), or serine (Ser, S). The 6th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably valine (Val, V), leucine (Leu, L), or isoleucine (Ile, I). The 9th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably isoleucine (Ile, I), valine (Val, V), phenylalanine (Phe, F), methionine (Met, M), or leucine (Leu, L). The 12th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably glycine (Gly, G) or alanine (Ala, A). The 15th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably leucine (Leu, L), valine (Val, V), or isoleucine (Ile, I). The 17th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably isoleucine (Ile, I), valine (Val, V), or methionine (Met, M). The 18th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably glycine (Gly, G) or alanine (Ala, A). The 22nd amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably aspartic acid (Asp, D) or histidine (His, H). The 26th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably valine (Val, V) or isoleucine (Ile, I). The 27th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 2 is preferably leucine (Leu, L), methionine (Met, M), or isoleucine (Ile, I). More specifically, a consensus sequence comprising the amino acid sequence shown in SEQ ID NO: 2 is preferably SGXT(G/A/S)(V/L/I)XX(I/V/F/M/L)XX(G/A)XX(L/V/I)X(I/V/M)(A/G)NXG(D/H)SRA(V/I)(L/M/I). In such amino acid sequence, pluralities of amino acids in parentheses represent possible variations of amino acid residues at the relevant positions. Also, in the following amino acid sequences, "X" means that any amino acid residue may be present at the relevant

position.

[0032]

Here, the amino acid sequence shown in SEQ ID NO: 3, an amino acid residue denoted as "Xaa," may be any amino acid, and it is not limited to any particular amino acid. However, the 4th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably methionine (Met, M), valine (Val, V), or phenylalanine (Phe, F). The 5th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably serine (Ser, S), alanine (Ala, A), or threonine (Thr, T). The 7th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably alanine (Ala, A) or serine (Ser, S). The 8th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably phenylalanine (Phe, F), isoleucine (Ile, I), or valine (Val, V). The 14th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably lysine (Lys, K) or glutamic acid (Glu, E). The 18th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably valine (Val, V) or leucine (Leu, L). The 19th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably isoleucine (Ile, I) or valine (Val, V). The 23rd amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably glutamic acid (Glu, E), glutamine (Gln, Q), or aspartic acid (Asp, D). The 24th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably isoleucine (Ile, I), valine (Val, V), or phenylalanine (Phe, F). The 29th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably isoleucine (Ile, I), leucine (Leu, L), or valine (Val, V). The 30th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably serine (Ser, S), threonine (Thr, T), or asparagine (Asn, N). The 33rd amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably aspartic acid (Asp, D), asparagine (Asn, N), or histidine (His, H). The

35th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably phenylalanine (Phe, F) or tyrosine (Tyr, Y). The 36th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably leucine (Leu, L), isoleucine (Ile, I), valine (Val, V), phenylalanine (Phe, F), or methionine (Met, M). The 37th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably valine (Val, V), leucine (Leu, L), or isoleucine (Ile, I). The 38th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably leucine (Leu, L) or valine (Val, V). The 40th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably threonine (Thr, T) or serine (Ser, S). The 43rd amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably valine (Val, V), isoleucine (Ile, I), or methionine (Met, M). The 44th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably tryptophan (Trp, W) or phenylalanine (Phe, F). The 45th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably aspartic acid (Asp, D) or glutamic acid (Glu, E). The 47th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably leucine (Leu, L), isoleucine (Ile, I), or methionine (Met, M). The 48th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably serine (Ser, S), threonine (Thr, T), or proline (Pro, P). The 49th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably asparagine (Asn, N) or serine (Ser, S). The 52nd amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably valine (Val, V) or alanine (Ala, A). The 55th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably leucine (Leu, L), valine (Val, V), isoleucine (Ile, I), or methionine (Met, M). The 56th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is preferably isoleucine (Ile, I) or valine (Val, V). Preferably, an

example of the consensus sequence comprising the amino acid sequence shown in SEQ ID NO: 3 is more specifically GXA(M/V/F)(S/A/T)R(A/S)(F/I/V)GDXXX(K/E)XXG(V/L)(I/V)XXP(E/Q/D)(I/V/F)XXXX (I/L/V)(T/S)XX(D/N/H)X(F/Y)(L/I/V/F)(V/L/I)(L/V)A(T/S)DG(V/I/M)(W/F)(D/E)X(L/I/M) (S/T/P)(N/S)XX(V/A)XX(L/V/I/M)(I/V). In such amino acid sequence, pluralities of amino acids in parentheses represent possible variations of amino acid residues at the relevant positions. Also, in the following amino acid sequences, "X" means that any amino acid residue may be present at the relevant position.

[0033]

Here, the 20th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is more preferably alanine (Ala, A), serine (Ser, S), or cysteine (Cys, C). Also, the 50th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 3 is more preferably aspartic acid (Asp, D), glutamic acid (Glu, E), lysine (Lys, K), glutamine (Gln, Q), or asparagine (Asn, N).

[0034]

Variations of amino acid residues that can be present at given positions are determined based on the following reasons. As described in Reference (1) ("McKee Biochemistry," 3rd ed., Chapter 5 Amino Acid·Peptide Protein 5.1 Amino Acid; editorial supervisor: Atsushi Ichikawa; translation supervisor: Shinichi Fukuoka; publisher: Ryosuke Sone; publishing office: Kagaku-Dojin Publishing Company, INC <http://www.kagakudojin.co.jp/profile/english.html>, ISBN4-7598-0944-9), it is well known that amino acids are classified based on side chains having similar properties (e.g., chemical properties and physical sizes). Also, it is well known that molecular evolutionary substitution frequently takes place among amino acid residues classified in a given group, while retaining protein activity. Based on these concepts, a substitution (mutation) score matrix for amino acid residues (BLOSUM: Blocks of Amino Acid Substitution Matrix) is proposed in Fig. 2 of Reference (2): Henikoff S., Henikoff J. G., Amino-acid substitution

matrices from protein blocks, Proc. Natl. Acad. Sci. U.S.A., 89, 10915-10919 (1992) and is broadly used. Reference (2) is based on a finding that amino acid substitutions that take place among amino acids with side chains having similar chemical properties result in less structural or functional changes in the entire protein. According to References (1) and (2) above, amino acid side chain groups to be used in multiple alignment can be considered based on indices such as chemical properties and physical sizes. They are shown as amino acid groups with a score of 0 or higher and preferably as amino acid groups with a score of 1 or higher through the use of the score matrix (BLOSUM) disclosed in Reference (2). Typical groups are the following 8 groups. Further precisely grouped amino acid groups may be amino acid groups with a score of 0 or higher, preferably a score of 1 or higher, and further preferably a score of 2 or higher.

[0035]

1) Aliphatic hydrophobic amino acid group (ILMV group)

This group is a group of amino acids having aliphatic hydrophobic side chains, among neutral nonpolar amino acids disclosed in Reference (1) above, which is composed of V (Val, valine), L (Leu, leucine), I (Ile, isoleucine), and M (Met, methionine). Among amino acids classified as neutral nonpolar amino acids according to Reference (1), FGACWP is not included in this "aliphatic hydrophobic amino acid group" because of the following reasons: G (Gly, glycine) and A (Ala, alanine) are the same size as that of or smaller in size than a methyl group and have weak non polar effects; C (Cys, cysteine) may play an important role in S-S bonds and has a property of forming a hydrogen bond with an oxygen atom or a nitrogen atom; F (Phe, phenylalanine) and W (Trp, tryptophan) have side chains with significantly large molecular weights and have strong aromatic effects; P (Pro, proline) has strong imino acid effects, so as to fix the angle of the main chain of the polypeptide.

[0036]

2) Group having hydroxymethylene group (ST group)

This group is a group of amino acids (from among neutral polar amino acids) having

hydroxymethylene groups in side chains, which is composed of S (Ser, serine) and T (Thr, threonine). Hydroxy groups existing in the side chains of S and T constitute sugar-binding sites, so that these sites are often important for a polypeptide (protein) to have specific activity.

[0037]

3) Acidic amino acid (DE group)

This group is a group of amino acids having acidic carboxyl groups in side chains, which is composed of D (Asp, aspartic acid) and E (Glu, glutamic acid).

[0038]

4) Basic amino acid (KR group)

This group is a group of basic amino acids, which is composed of K (Lys, lysine) and R (Arg, arginine). These K and R are positively charged within a wide pH range and have basic properties. On the other hand, H (His, histidine) classified in basic amino acids is almost never ionized at pH 7, so that H is not classified in this group.

[0039]

5) Methylene group = polar group (DHN group)

This group is characterized in that: in all cases, a methylene group as a side chain binds to an α-carbon element beyond which a polar group is present; and the physical sizes of methylene groups (nonpolar groups) closely resemble from each other. This group is composed of N (Asn, asparagine; polar group is an amide group), D (Asp, aspartic acid; polar groups are carboxyl groups), and H (His, histidine; polar groups are imidazole groups).

6) Dimethylene group = polar group (EKQR group)

This group is characterized in that: in all cases, linear hydrocarbon having a length longer than that of a dimethylene group binds as a side chain to an α-carbon element, beyond which a polar group is present; and the physical sizes of dimethylene groups that are nonpolar groups closely resemble from each other. This group is composed of E (Glu, glutamic acid, polar group is a carboxyl group), K (Lys, lysine; polar groups are amino groups), Q (Gln,

glutamine; polar groups are amide groups), and R (Arg, arginine; polar groups are imino groups and amino groups).

[0040]

7) Aromatic series (FYW group)

This group is a group of aromatic amino acids having benzene nuclei in the side chains and characterized by having chemical properties unique in aromatic series. This group is composed of F (Phe, phenylalanine), Y (Tyr, tyrosine), and W (Trp, tryptophan).

[0041]

8) Ring & polar (HY group)

This group is a group of amino acids having both ring structures in the side chains and polarity, which is composed of H (H, histidine; Both ring structures and polar groups are imidazole groups), and Y (Tyr, tyrosine; Ring structures are benzene nuclei and polar groups are hydroxy groups).

[0042]

As described above, it is understood that: in the given amino acid sequences shown in SEQ ID NOS: 1-3, an amino acid residue denoted as Xaa may be any amino acid; or amino acid residues denoted as Xaa may be substituted with each other within the above groups 1)-8). Hence, in the present invention, the protein phosphatase 2C gene to be introduced into a plant or subjected to modification of an expression control region may be a protein phosphatase 2C gene from any plant, as long as it has the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order.

[0043]

More specifically, examples of an *Arabidopsis thaliana* protein phosphatase 2C-coding gene having the 3 consensus sequences (comprising the amino acid sequences shown in SEQ ID NOS: 1-3) from the N-terminal side in such order include At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and

At3g06270. In the present invention, at least one type of gene selected from the gene group is introduced into a plant or an expression control region of an endogenous gene corresponding to the gene is modified. Particularly in the present invention, it is preferable to introduce at least one type of gene selected from among At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7 into a plant, or to modify an expression control region of an endogenous gene corresponding to the gene. Particularly, in the present invention, it is more preferable to introduce at least one type of gene selected from among At3g16800, At3g05640, and At5g27930-AtPP2C6-7 into a plant, or to modify the expression control region of the endogenous gene corresponding to the gene. It is most preferable to introduce the gene specified by At3g05640 into a plant or to modify an expression control region of an endogenous gene corresponding to the gene.

[0044]

In addition, Fig. 2 shows the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program (that can be used with the DDBJ of the National Institute of Genetics (http://clustalw.ddbj.nig.ac.jp/top-j.html)) for amino acid sequences encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7 (amino acid (sequence) substitution matrix used herein is default matrix, BLOSUM (Blocks of Amino Acid Substitution Matrix)).

[0045]

That is, Fig. 2 shows the 3 consensus sequences in protein phosphatase 2C encoded by At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, and At5g27930-AtPP2C6-7. Regions denoted as I-III in Fig. 2 are subjected with an ortholog of a rice-derived protein phosphatase 2C gene (described later) to alignment analysis, so that the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 above can be defined as the 3

consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 48-55, respectively.

[0046]

The consensus sequence shown in SEQ ID NO: 48 is more specifically (L/F)CG(V/I/M)FDGHGXXGXX(V/I)(S/A)(K/R)XV. The consensus sequence shown in SEQ ID NO: 49 is more specifically SGXT(G/A/S)(V/L)XX(I/V/F/L)XX(G/A)XX(L/V/I)X(I/V/M)(A/G)NXG(D/H)SRA(V/I)(L/M/I). The consensus sequence shown in SEQ ID NO: 50 is more specifically GLA(M/V)(S/A)R(A/S)(F/L)GDXX(L/I/V)KX(Y/F/H)G(V/L)(I/V)XXP(E/Q/D)(I/V/F)XXXX(I/L/V)(T/S)XXDX(F/Y)(L/I/V/M)(V/L/I)LA(T/S)DG(V/I/M)WDX(L/I/M/V)(S/T)NX(E/D)(V/A)XX(L/V/I)(I/V).

[0047]

In addition, in such amino acid sequences, pluralities of amino acids in parentheses represent possible variations of amino acid residues at the relevant positions. Also, in these amino acid sequences, "X" means that any amino acid residue may be present at the relevant position.

[0048]

Here, the 9th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 49 is more preferably isoleucine (Ile, I), valine (Val, V), or phenylalanine (Phe, F). Also, the 11th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 49 is more preferably glutamine (Gln, Q) or histidine (His, H). Moreover, the 13th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 49 is more preferably lysine (Lys, K), glutamic acid (Glu, E), serine (Ser, S), glutamine (Gln, Q), aspartic acid (Asp, D), or asparagine (Asn, N).

[0049]

Here, the 7th amino acid residue from the N-terminal side in the amino acid sequence

shown in SEQ ID NO: 50 is more preferably alanine (Ala, A). Also, the 8th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably phenylalanine (Phe, F). Moreover, the 11th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably phenylalanine (Phe, F) or tyrosine (Tyr, Y). Furthermore, the 13th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably leucine (Leu, L) or isoleucine (Ile, I). Moreover, the 15th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably aspartic acid (Asp, D), serine (Ser, S), or glutamic acid (Glu, E). Furthermore, the 20th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably serine (Ser, S), alanine (Ala, A), or cysteine (Cys, C). Moreover, the 27th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably histidine (His, H) or arginine (Arg, R). Furthermore, the 34th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably glutamine (Gln, Q), glutamic acid (Glu, E), or histidine (His, H). Furthermore, the 36th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably leucine (Leu, L), isoleucine (Ile, I), or valine (Val, V). Furthermore, the 47th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably leucine (Leu, L), isoleucine (Ile, I), or valine (Val, V). Furthermore, the 50th amino acid residue from the N-terminal side in the amino acid sequence shown in SEQ ID NO: 50 is more preferably lysine (Lys, K), glutamic acid (Glu, E), glutamine (Gln, Q), aspartic acid (Asp, D), or asparagine (Asn, N).

[0050]

As examples, the nucleotide sequence of the coding region in the gene specified by At3g05640 is shown in SEQ ID NO: 4 and the amino acid sequence of protein phosphatase 2C encoded by the gene specified by At3g05640 is shown in SEQ ID NO: 5.

[0051]

Also, in the present invention, genes functionally equivalent to genes listed above may also be introduced into a plant or the expression control regions of endogenous genes corresponding to the genes may be modified. Here, the term "functionally equivalent gene" refers to, for example, a gene (from an organism other than *Arabidopsis thaliana*) that: has the 3 consensus sequences (preferably, the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 48-50. The same applies to the following) comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order; and encodes protein phosphatase 2C. Also, the term "functionally equivalent gene" refers to a gene that encodes a protein having protein phosphatase 2C activity. The term "protein phosphatase 2C activity" refers to Mg^{2+}- or Mn^{2+}-dependent serine/threonine phosphatase (Ser/Thr phosphatase) activity. Therefore, whether or not a gene encodes a protein having protein phosphatase 2C activity can be confirmed by examining whether or not the gene product has serine/threonine phosphatase activity in the presence of Mg^{2+} or Mn^{2+}. Conventionally known techniques can be appropriately employed for determining serine/threonine phosphatase activity. For example, a commercially available activity determination kit ProFluor (registered trademark) Ser/Thr Phosphatase Assay (Promega) can be used.

[0052]

Here, example of organisms other than *Arabidopsis thaliana* is not limited. For example, rice (*Oryza sativa*) is included. Specifically, an example of a functionally equivalent gene is a rice Os05g0358500 gene. The nucleotide sequence of a coding region of the Os05g0358500 gene is shown in SEQ ID NO: 6 and the amino acid sequence of the protein encoded by the gene is shown in SEQ ID NO: 7. Also, examples of the above-mentioned rice-derived functionally equivalent gene include Os11g0109000 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 8 and 9, respectively), Os12g0108600 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 10 and 11, respectively), Os02g0471500 (the nucleotide sequence

and the amino acid sequence are shown in SEQ ID NOS: 12 and 13, respectively), Os04g0321800 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 14 and 15, respectively), Os11g0417400 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 16 and 17, respectively), Os07g0566200 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 18 and 19, respectively), Os08g0500300 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 20 and 21, respectively), Os02g0224100 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 22 and 23, respectively), and Os02g0281000 (the nucleotide sequence and the amino acid sequence are shown in SEQ ID NOS: 51 and 52, respectively).

[0053]

Moreover, examples of the above-mentioned functionally equivalent genes from plants other than *Arabidopsis thaliana* and rice include genes (UniProt data base Accession Nos. A9P973, A9PFS0, and A9P7U4) from black cottonwood (*Populus trichocarpa*), genes (UniProt data base Accession Nos. A7PRZ8, A7Q8H4, A7PV59, A5C3B0, A5BF43, A7QFG6, A7P4H7, A5C0C9, A5AP53, A7QQF9, and A5BDP5) from European grape (*Vitis vinifera*), genes (UniProt data base Accession Nos. Q2HW33 and Q4L0F8) from *Medicago truncatula* (*Medicago truncatula*), a gene (GenBank data base Accession No. AY651248) from alfalfa (*Medicago sativa*), genes (UniProt data base Accession Nos. A9SE70, A9SE69, and A9RFU1) from *Physcomitrella patens* (*Physcomitrella patens*), a gene (UniProt data base Accession No. 2511453C) from ice plant (*Mesembryanthemum crystallinum*), a gene (UniProt data base Accession No. A8HQG8) from *Chlamydomonas reinhardtii* (*Chlamydomonas reinhardtii*), genes (GenBank data base Accession Nos. BT024031, BT017414, and BT024134) from corn (*Zea mays*), genes (GenBank data base Accession Nos. AC189312 and AC189579) from rapeseed (*Brassica rapa*), genes (GenBank data base Accession Nos. AP009550, AP009302, and AP009278) from tomato (*Solanum lycopersicum*), a gene (GenBank data base Accession No. AC182571) from monkey flower (*Mimulus guttatus*), and

a gene (GenBank data base Accession No. AP006489) from monocellular red alga (*Cyanidioschyzon merolae*).

[0054]

In these plants other than *Arabidopsis thaliana*, which are represented by the above examples, a gene encoding protein phosphatase 2C that has the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order can be easily searched for and/or identified from a known database such as GenBank based on the above-listed nucleotide sequence of *Arabidopsis thaliana*-derived protein phosphatase 2C gene or amino acid sequence of protein phosphatase 2C.

[0055]

In addition, a protein phosphatase 2C gene in the present invention is not limited to the above described protein phosphatase 2C genes comprising the nucleotide sequences and the amino acid sequences shown in SEQ ID NOS: 4-23. Hence, the protein phosphatase 2C gene may be a gene that contains an amino acid sequence having a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acid sequences with respect to the amino acid sequences shown in odd numbers of SEQ ID NOS: 4-23, and, has protein phosphatase 2C activity. Here the term "a plurality of amino acids" refers to 1 to 20, preferably 1 to 10, more preferably 1 to 7, further preferably 1 to 5, and particularly preferably 1 to 3 amino acids, for example. In addition, amino acid deletion, substitution, or addition can be performed by modifying a nucleotide sequence encoding the above protein phosphatase 2C gene by a technique known in the art. Mutation can be introduced into a nucleotide sequence by a known technique such as the Kunkel method or the Gapped duplex method or a method based thereof. For example, mutation is introduced with a mutagenesis kit using site-directed mutagenesis (e.g., Mutant-K or Mutant-G (both are trade names of Takara Bio)) or the like, or a LA PCR *in vitro* Mutagenesis series kit (trade name, Takara Bio). Also, a mutagenesis method may be: a method using a chemical mutation agent represented by EMS (ethyl methanesulfonate), 5-bromouracil, 2-aminopurine,

hydroxylamine, N-methyl-N'-nitro-N nitrosoguanidine, or other carcinogenic compounds; or a method that involves radiation treatment or ultraviolet [UV] treatment typically using X-rays, alpha rays, beta rays, gamma rays, an ion beam, or the like.

[0056]

Also, protein phosphatase 2C genes to be used herein may be genes homologous to the protein phosphatase 2C genes comprising the nucleotide sequences and the amino acid sequences shown in SEQ ID NOS: 4-23. Here, the term "homologous gene" generally refers to a gene that has evolutionarily branched off from a common ancestor gene, including a homologous gene (ortholog) of 2 types of species and a homologous gene (paralog) generated by overlapping branching that takes place within the same species. In other words, the above term "functionally equivalent gene" refers to a homologous gene such as an ortholog or a paralog. Furthermore, the above term "functionally equivalent gene" may also refer to a gene that does not evolve from a common gene, but simply has analogous functions.

[0057]

Examples of genes analogous to the protein phosphatase 2C genes comprising the nucleotide sequences and the amino acid sequences shown in SEQ ID NOS: 4-23 include genes encoding proteins having: amino acid sequences that have 70% or more, preferably 80% or more, more preferably 90% or more, and most preferably 95% or more similarity to these amino acid sequences; the 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order; and protein phosphatase 2C activity. Here, the value of similarity refers to a value that can be found based on default setting using a computer program mounted with a BLAST (Basic Local Alignment Search Tool) program and a database containing gene sequence information.

[0058]

Also, genes analogous to protein phosphatase 2C genes comprising the nucleotide sequences and the amino acid sequences shown in SEQ ID NOS: 4-23 can be identified by, when the plant genome information remains unclarified, extracting the genome from a target

plant or constructing a cDNA library for a target plant and then isolating a genomic region or cDNA hybridizing under stringent conditions to at least a portion of the protein phosphatase 2C genes comprising the nucleotide sequences and the amino acid sequences shown in SEQ ID NOS: 4-23. Here, the term "stringent conditions" refers to conditions under which namely a specific hybrid is formed, but a non-specific hybrid is never formed. For example, such conditions comprise hybridization at 45°C with 6 x SSC (sodium chloride/sodium citrate), followed by washing at 50°C to 65°C with 0.2-1 x SSC and 0.1% SDS. Alternatively, such conditions comprise hybridization at 65°C to 70°C with 1 x SSC, followed by washing at 65°C to 70°C with 0.3 x SSC. Hybridization can be performed by a conventionally known method such as a method described in J. Sambrook et al. Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory (1989).

[0059]

Examples of a technique for introducing such protein phosphatase 2C gene into a plant include a technique for introducing an expression vector in which an exogenous protein phosphatase 2C gene is arranged under control of a promoter that enables expression in a target plant. Examples of a technique for modifying an expression control region of an endogenous gene corresponding to the gene include a technique for modifying a promoter of an endogenous protein phosphatase 2C gene in a target plant.

[0060]

A preferred example is a technique for introducing an expression vector in which the above protein phosphatase 2C gene is arranged under control of a promoter that enables expression in a target plant.

[0061]

Glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene

In the present invention, the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene is a gene which has activity that is controlled by its binding to glutathione and encodes an enzyme having activity of catalyzing a reaction (reversible

reaction) for conversion of fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate within a plastid such as chloroplast. The glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene may be derived from any plant, as long as it encodes an enzyme having the above activity, or may also be a mutant gene resulting from introduction of mutation into a gene isolated from a plant.

[0062]

As an example, *Arabidopsis thaliana*-derived glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene can be used herein. The *Arabidopsis thaliana*-derived glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene is disclosed as an FBA1 gene in WO2007/091634. The nucleotide sequence of the coding region in the FBA1 gene is shown in SEQ ID NO: 31, and the amino acid sequence of glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase encoded by the FBA1 gene is shown in SEQ ID NO: 32. Also, the FBA1 gene in *Arabidopsis thaliana* is also referred to as an At2g01140 gene.

[0063]

Also, in the present invention, a gene functionally equivalent to the above At2g01140 gene may be introduced into a plant or an expression control region of an endogenous gene corresponding to the gene may be modified. Here, the term "functionally equivalent gene" refers to a gene from an organism other than *Arabidopsis thaliana*, including a gene having glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase activity, as an example. Also, fructose 1,6-bisphosphate aldolase activity can be measured by causing a protein to be measured for activity to act in a buffer containing fructose 1,6-bisphosphate as a substrate and then measuring the thus generated dihydroxyacetone phosphate and/or glyceraldehyde-3-phosphate. Alternatively, fructose 1,6-bisphosphate aldolase activity can also be measured as follows. Specifically, first, a protein to be measured for activity is caused to act in a buffer containing fructose 1,6-bisphosphate as a substrate. Triose phosphate isomerase is caused to act on the thus generated glyceraldehyde-3-phosphate, so

that dihydroxyacetone phosphate is generated. When the dihydroxyacetone phosphate is altered to glycerol-3-phosphate in the presence of glycerol-3-phosphate dehydrogenase, NADH is converted to β-nicotinamide adenine dinucleotide (oxidized) (NAD). Hence, NADH-derived 340-nm absorbance decreases. Therefore, the protein to be measured for its aldolase activity can be evaluated by measuring the reduction rate of NADH.

[0064]

Also, the glutathione binding property of a protein to be evaluated can be evaluated based on the presence or the absence of a glutathione binding sequence in the amino acid sequence of the protein or can also be evaluated by causing a protein to be evaluated to act in the presence of, glutathione transferase and glutathione and then measuring the presence or the absence of the binding of glutathione.

[0065]

Moreover, whether a protein to be evaluated is of a plastid type can be evaluated based on the presence or the absence of a transit peptide sequence in the amino acid sequence of the protein, or can also be evaluated by producing a plant through introduction of a gene encoding a fusion protein of the protein and a reporter such as a fluorescent protein, detecting the reporter, and then detecting the localization of the plastid of the protein.

[0066]

Here, examples of organisms other than *Arabidopsis thaliana* include, but are not limited to, rice (*Oryza sativa*). Specifically, an example of a functionally equivalent gene is a rice Os01g0118000 gene. The amino acid sequence of the protein encoded by the Os01g0118000 gene is shown in SEQ ID NO: 43.

[0067]

Furthermore, examples of the above functionally equivalent genes from plants other than *Arabidopsis thaliana* and rice include a European grape (*Vitis vinifera*)-derived gene (NCBI Entrez Protein database Accession No. CAO42215 (SEQ ID NO: 40)), a castor-oil plant (*Ricinus communis*)-derived gene (NCBI Entrez Protein database Accession No.

EEF36097 (SEQ ID NO: 39)), black cottonwood (*Populus trichocarpa*)-derived genes (NCBI Entrez Protein database Accession Nos. EEE88847 (SEQ ID NO: 38), EEF02079 (SEQ ID NO: 36) and ABK94899 (SEQ ID NO: 37)), Sitka Spruce (*Picea sitchensis*)-derived genes (NCBI Entrez Protein database Accession No. ABK24568 (SEQ ID NO: 35), ABK24286 (SEQ ID NO: 33), and ABK25226 (SEQ ID NO: 34)), and corn (*Zea mays*)-derived genes (NCBI Entrez Protein database Accession No. ACG47464 (SEQ ID NO: 41) and ACG47669 (SEQ ID NO: 42)).

[0068]

In these plants other than *Arabidopsis thaliana*, which are represented by the above examples, a gene having glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase activity can be easily searched for and/or identified from a known database such as GenBank based on the nucleotide sequence of above-listed *Arabidopsis thaliana*-derived FBA1 gene (At2g01140gene) or the amino acid sequence of the protein encoded by the gene.

[0069]

Fig. 3 shows the results of alignment analysis using a CLUSTAL W (1.83) multiple sequence alignment program (which can be used with the DDBJ of the National Institute of Genetics (http://clustalw.ddbj.nig.ac.jp/top-j.html)) for the amino acid sequences shown in SEQ ID NOS: 32-43 (with the amino acid (sequence) substitution matrix used herein being a default matrix known as BLOSUM (Blocks of Amino Acid Substitution Matrix)). As shown in Fig. 3, glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase genes exhibited very high homology. Also, Fig. 4 shows the result of creating a phylogenetic tree of the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase genes of the amino acid sequences shown in SEQ ID NOS: 32-43, *Arabidopsis thaliana* fructose 1,6-bisphosphate aldolase genes (FBA2 gene (At4g38970) and FBA3 gene (At2g21330)), and tobacco fructose 1,6-bisphosphate aldolase gene (BAA77604). As shown in the frame (broken line) in Fig. 4, the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase genes of the amino acid sequences shown in SEQ ID NOS: 32-43 form a group differing from those of the

Arabidopsis thaliana FBA2 gene and FBA3gene and the tobacco BAA77604 gene.

[0070]

In addition, the fructose 1,6-bisphosphate aldolase proteins encoded by the *Arabidopsis thaliana* FBA2 gene and FBA3 gene do not have a characteristic of exhibiting fructose 1,6-bisphosphate aldolase activity due to glutathione.

[0071]

In the present invention, examples of a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene are not limited to the above gene encoding the nucleotide sequence shown in SEQ ID NO: 31 and genes encoding proteins comprising the amino acid sequences shown in SEQ ID NOS: 32-43. Specifically, examples of the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase genes comprise amino acid sequences that have a deletion(s), a substitution(s), an addition(s), or an insertion(s) of one or a plurality of amino acid sequences with respect to the amino acid sequences shown in SEQ ID NOS: 32-43, and, have glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase activity. Here, the term "a plurality of amino acids" refers to 1 to 20 amino acids, preferably 1 to 10 amino acids, more preferably 1 to 7 amino acids, further preferably 1 to 5 amino acids, and particularly preferably 1 to 3 amino acids. In addition, for deletion, substitution, or addition of an amino acid(s), techniques disclosed in the above column, "Protein phosphatase 2C gene" can be applied.

[0072]

Also, the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase genes may be homologous to genes encoding the nucleotide sequence shown in SEQ ID NO: 31 and the proteins comprising the amino acid sequences shown in SEQ ID NOS: 32-43. Here, the term "homologous gene" generally refers to a gene that has evolutionarily branched off from a common ancestor gene, including a homologous gene (ortholog) of 2 types of species and a homologous gene (paralog) generated by overlapping branching that takes place within the same species. In other words, the above term "functionally equivalent gene" refers to a

homologous gene such as an ortholog or a paralog. Furthermore, the above term "functionally equivalent gene" may also refer to a gene that does not evolve from a common gene, but simply has analogous functions.

[0073]

Examples of genes analogous to the gene encoding the nucleotide sequence shown in SEQ ID NO: 31 and the genes encoding the proteins comprising the amino acid sequences shown in SEQ ID NOS: 32-43 include genes encoding proteins that have amino acid sequences having 70% or more, preferably 80% or more, more preferably 90% or more, and most preferably 95% or more similarity with respect to the amino acid sequences of SEQ ID NOS: 32-43 and have glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase activity. Here, the values for similarity are obtained through application of techniques disclosed in the above column "Protein phosphatase 2C gene".

[0074]

Also, genes analogous to a gene containing the nucleotide sequence shown in SEQ ID NO: 31 and the genes encoding the proteins comprising the amino acid sequences shown in SEQ ID NOS: 32-43 can be identified by, when the plant genome information remains unclarified, extracting the genome from a target plant or constructing a cDNA library for a target plant and then isolating a genomic region or cDNA hybridizing under stringent conditions to at least a portion of a gene containing the nucleotide sequence shown in SEQ ID NO: 31 and the genes encoding the proteins comprising the amino acid sequences shown in SEQ ID NOS: 32-43. Here, the term "stringent conditions" refers to conditions as disclosed in the above column "Protein phosphatase 2C gene". Techniques as disclosed in the above column "Protein phosphatase 2C gene" can be applied to hybridization.

[0075]

As a technique for introducing the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene into a plant or modifying an expression control region of an endogenous gene corresponding to the gene, techniques disclosed in the above column

"Protein phosphatase 2C gene" are applicable.

[0076]

Expression vector

An expression vector is constructed to contain a promoter that enables expression within plants and the above described protein phosphatase 2C gene and glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene. In addition, an expression vector containing the protein phosphatase 2C gene and an expression vector containing the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene may be separately prepared.

[0077]

As a vector serving as a mother body for an expression vector, various conventionally known vectors can be used. For example, plasmids, phages, cosmids, or the like can be used and such vector can be appropriately selected depending on plant cells into which it is introduced and introduction methods. Specific examples of such vector include pBR322, pBR325, pUC19, pUC119, pBluescript, pBluescriptSK, and pBI vectors. Particularly, when a method for introduction of a vector into a plant uses Agrobacterium, a pBI binary vector is preferably used. Specific examples of such pBI binary vector include pBIG, pBIN19, pBI101, pBI121, and pBI221.

[0078]

A promoter to be used herein is not particularly limited, as long as it enables expression of a protein phosphatase 2C gene and a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene within a plant. Any known promoter can be appropriately used. Examples of such promoter include a cauliflower mosaic virus 35S promoter (CaMV35S), various actin gene promoters, various ubiquitin gene promoters, a nopaline synthase gene promoter, a tobacco PR1a gene promoter, a tomato ribulose 1,5-bisphosphate carboxylase·oxidase small subunit gene promoter, and a napin gene promoter. Of these, a cauliflower mosaic virus 35S promoter, an actin gene promoter, or a ubiquitin gene promoter

can be more preferably used. The use of each of the above promoters enables strong expression of any gene when it is introduced into plant cells.

[0079]

Also, a promoter having functions of causing site-specific expression in a plant can also be used herein. As such promoter, any conventionally known promoter can be used. When the above described protein phosphatase 2C gene or glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene is site-specifically expressed using such promoter, a plant organ in which the gene is expressed can be more increased than wild-type plant organs.

[0080]

In addition, an expression vector may further contain other DNA segments in addition to a promoter, the above protein phosphatase 2C gene and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase 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 above recombinant expression vector may further have a T-DNA region. A T-DNA region can enhance efficiency for gene introduction particularly when the above recombinant expression vector is introduced into a plant using Agrobacterium.

[0081]

A transcription terminator is not particularly limited, as long as it has functions as a transcription termination site and may be any known transcription terminator. For example, specifically, a transcription termination region (Nos terminator) of a nopaline synthase gene, a transcription termination region (CaMV35S terminator) of cauliflower mosaic virus 35S, or the like can be preferably used. Of them, the Nos terminator can be more preferably used. In the case of the above recombinant vector, a phenomenon such that an unnecessarily long transcript is synthesized and that a strong promoter decreases the number of copies of a transcript after introduction into plant cells can be prevented by arranging a transcription terminator at an appropriate position.

[0082]

As a transformant selection marker, a drug resistance gene can be used, for example. Specific examples of such drug resistance gene include drug resistance genes against hygromycin, bleomycin, kanamycin, gentamicin, chloramphenicol, and the like. Transformed plants can be easily selected by selecting plants that can grow in medium containing the above antibiotics.

[0083]

An example of a nucleotide sequence for increasing translation efficiency is an omega sequence from tobacco mosaic virus. This omega sequence is arranged in an untranslated region (5'UTR) of a promoter, so that the translation efficiency of the fusion gene can be increased. As such, the recombinant expression vector can contain various DNA segments depending on purposes.

[0084]

A method for constructing a recombinant expression vector is not particularly limited. To an appropriately selected vector serving as a mother body, the above promoter, the above protein phosphatase 2C gene and/or the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene, a transcription repressor converting polynucleotide, and if necessary, the above other DNA segments may be introduced in an predetermined order. For example, the above protein phosphatase 2C gene or the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene and a promoter (and, if necessary, a transcription terminator or the like) are linked to construct an expression cassette and then the cassette may be introduced into a vector. In construction of an expression cassette, for example, cleavage sites of DNA segments are prepared to have protruding ends complementary to each other and then performing a reaction with a ligation enzyme, making it possible to specify the order of the DNA segments. In addition, when an expression cassette contains a terminator, DNA segments may be arranged in the following order from upstream: a promoter, the above protein phosphatase 2C gene or the above

glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene, and a terminator. Also, reagents for construction of an expression vector (that is, types of restriction enzymes, ligation enzymes, and the like) are also not particularly limited. Hence, commercially available reagents can be appropriately selected and used.

[0085]

Also, a method for replicating (a method for producing) the above expression vector is not particularly limited and conventionally known replication methods can be used herein. In general, such expression vector may be replicated within *Escherichia coli* as a host. At this time, preferred types of *Escherichia coli* may be selected depending on the types of vector.

[0086]

Transformation

The above-described expression vector is introduced into a target plant by a general transformation method. A method for introducing an expression vector into plant cells (transformation method) is not particularly limited. Conventionally known appropriate introduction methods can be used depending on plant cells. Specifically, a method using Agrobacterium or a method that involves direct introduction into plant cells can be used, for example. As a method using Agrobacterium, a method 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., or a method described in Zyprian E, Kado Cl, 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. can be employed, for example.

[0087]

As a method for directly introducing an expression vector into plant cells, microinjection, electroporation, a polyethylene glycol method, a particle gun method, protoplast fusion, a calcium phosphate method, or the like can be employed.

[0088]

Also, when a method for directly introducing DNA into plant cells is employed, DNA that can be used herein contains transcriptional units required for the expression of a target gene, such as a promoter and a transcription terminator, and a target gene. Vector functions are not essential in such case. Moreover, a DNA that contains a protein coding region alone of a target gene having no transcriptional unit may be used herein, as long as it is integrated into a host's transcriptional unit and then the target gene can be expressed.

[0089]

Examples of plant cells into which the above expression vector or an expression cassette containing no expression vector, but a target gene is introduced include cells of each tissue of plant organs such as flowers, leaves, and roots, calluses, and suspension-cultured cells. At this time, an appropriate expression vector may be constructed according to the types of plant to be produced or a versatile expression vector may be constructed in advance and then introduced into plant cells.

[0090]

Plants into which an expression vector is introduced or in other words, plants required to increase the production of biomass are not particularly limited. Specifically, through introduction of the above-described protein phosphatase 2C gene and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene, effects of increasing the production of biomass can be expected for all plants. Examples of target plants include, but are not limited to, dicotyledons and monocotyledons, such as plants (see below) belonging to the families Brassicaceae, Gramineae, Solanaceae, Leguminosae, Salicaceae, and the like.

[0091]

Family Brassicaceae: *Arabidopsis thaliana* (*Arabidopsis thaliana*), rapeseed (*Brassica rapa*, *Brassica napus*, *Brassica campestris*), cabbage (*Brassica oleracea* var. capitata), napa (*Brassica rapa* var. pekinensis), ging-geng-cai (*Brassica rapa* var. chinensis), turnip (*Brassica rapa* var. rapa), turnip greens (*Brassica rapa* var. hakabura), potherb mustard

(*Brassica rapa* var. lancinifolia), komatsuna (*Brassica rapa* var. peruviridis), pak choi (*Brassica rapa* var. chinensis), daikon (*Raphanus sativus*), Japanese horseradish (*Wasabia japonica*), and the like.

[0092]

Family Solanaceae: tobacco (*Nicotiana tabacum*), eggplant (*Solanum melongena*), potato (*Solaneum tuberosum*), tomato (*Lycopersicon lycopersicum*), chile pepper (*Capsicum annuum*), petunia, and the like.

[0093]

Family Leguminosae: soy (*Glycine max*), pea (*Pisum sativum*), broad bean (*Vicia faba*), Wisteria (*Wisteria floribunda*), peanuts (*Arachis hypogaea*), bird's foot trefoil (*Lotus corniculatus var. japonicus*), common bean (*Phaseolus vulgaris*), azuki bean (*Vigna angularis*), acacia, and the like.

[0094]

Family Asteraceae: florists' daisy (*Chrysanthemum morifolium*), sunflower (*Helianthus annuus*), and the like.

[0095]

Family Arecaceae: oil palm (*Elaeis guineensis*, *Elaeis oleifera*), coconut (*Cocos nucifera*), date palm (*Phoenix dactylifera*), copernicia, and the like.

[0096]

Family Anacardiaceae: wax tree (*Rhus succedanea*), cashew nut (*Anacardium occidentale*), lacquer tree (*Toxicodendron vernicifluum*), mango (*Mangifera indica*), pistachio (*Pistacia vera*), and the like.

[0097]

Family Cucurbitaceae: pumpkin (*Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*), cucumber (*Cucumis sativus*), snake gourd (*Trichosanthes cucumeroides*), gourd (*Lagenaria siceraria* var. gourda), and the like.

[0098]

Family Rosaceae: almond (*Amygdalus communis*), rose (Rosa), strawberry (Fragaria), cherry (Prunus), apple (*Malus pumila* var. domestica), and the like.

[0099]

Family Caryophyllaceae: carnation (*Dianthus caryophyllus*) and the like.

[0100]

Family Salicaceae: poplar (*Populus trichocarpa*, *Populus nigra*, or *Populus tremula*) and the like.

[0101]

Family Gramineae: corn (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), bamboo (*Phyllostachys*), sugarcane (*Saccharum officinarum*), napier grass (*Pennisetum pupureum*), erianthus (*Erianthus ravenae*), miscanthus (Japanese silver grass) (*Miscanthus virgatum*), sorghum (Sorghum) and switch grass (Panicum), and the like

[0102]

Family Liliaceae: tulip (Tulipa), lily (Lilium), and the like.

[0103]

Of these examples, energy crops such as sugarcane, corn, rapeseed, and sunflower, which can serve as raw materials for biofuel, may be preferable targets. This is because the costs for biofuel such as bioethanol, biodiesel, biomethanol, bioDME, bioGTL (BTL), and biobutanol can be reduced by increasing the production of biomass using energy crops.

[0104]

Also, as described above, protein phosphatase 2C genes that can be used in the present invention and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene can be isolated from various plants and used. Such protein phosphatase 2C genes and glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene can be appropriately selected and used, depending on the types of target plant required to increase the biomass production.

[0105]

Specifically, when a plant required to increase the biomass production is a monocotyledon, a protein phosphatase 2C gene that is isolated from a monocotyledon is preferably introduced. In particular, when a plant required to increase the biomass production is rice, the rice-derived protein phosphatase 2C gene (SEQ ID NO: 6) is preferably introduced. The above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene can be similarly selected depending on plants required to increase the biomass production.

[0106]

In addition, in the present invention, even when a plant required to increase the biomass production is a monocotyledon, a dicotyledon-derived protein phosphatase 2C gene or glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene may be introduced. Specifically, for example, the *Arabidopsis thaliana*-derived protein phosphatase 2C gene (SEQ ID NO: 4) and the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene (SEQ ID NO: 31) may be introduced into not only dicotyledons, but also a variety of plants that are classified as monocotyledons, so that the genes are expressed.

[0107]

Meanwhile, when an expression vector containing a protein phosphatase 2C gene and an expression vector containing a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene are separately prepared, transformation may be performed using both expression vectors, or a transformed plant may be obtained by transformation using one of the expression vectors, following which such transformed plant may be further transformed with the other expression vector. For example, a transformed plant is obtained using one of the expression vectors, self-fertilized seeds of the transformed plant are harvested, the fixation of the introduced genes in the progeny plants is confirmed, and thus the progeny plants can be transformed with the other expression vector.

[0108]

Also, a transformed plant introducing a protein phosphatase 2C gene and a transformed plant introducing a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene may be produced separately The thus separately produced transformed plants are crossed, and thus progeny plants having both the protein phosphatase 2C gene and the glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene can be obtained.

[0109]

Furthermore, through induction of mutation, introduction of a gene activating factor, and the like, plants in which the expression of the plants' own protein phosphatase 2C gene and glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene is activated may be selected.

[0110]

Other steps and methods

After the above transformation, a step of screening for proper transformants from plants can be performed by a conventionally known method. Such screening method is not particularly limited. For example, selection can be made based on drug resistance such as hygromycin resistance. Alternatively, after the growth of transformants, a transformant with a significant increase in biomass production compared with a wild type plant may be screened for by determining the weight of a plant itself or its arbitrary organ or tissue.

[0111]

Also, progeny plants can be obtained from transformed plants obtained by transformation according to a conventional method. Progeny plants retaining a trait resulting from the introduction of the above protein phosphatase 2C gene and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene or a trait resulting from the modification of an expression control region of endogenous genes corresponding to the genes are selected based on their amounts of biomass. Therefore, because of having the above trait, a stable plant line exhibiting the increased production of biomass can be produced. Also, plant cells or reproductive materials, such as seeds, fruits, stocks, calluses,

tubers, cut ears, or lumps, may be obtained from such a transformed plant or progeny plants thereof. Because of having the above trait, a stable plant line exhibiting the increased production of biomass can be mass-produced from such cells or materials.

[0112]

In addition, the plant of the present invention may include a matter comprising at least any one of adult plants, plant cells, plant tissues, calli, and seeds. That is, according to the present invention, any matter in a state that allows it to eventually grow to become a plant can be regarded as a plant. In addition, the above plant cells include plant cells in various forms. Examples of such plant cells include suspension-cultured cells, protoplasts, and leaf sections. As a result of proliferation/differentiation of such plant cells, a plant can be obtained. In addition, a plant can be reproduced from plant cells by a conventionally known method depending on the types of plant cells.

[0113]

As explained above, according to the present invention, plants capable of exhibiting the significantly increased production of biomass and/or seeds per plant compared with a wild-type plant can be provided by introducing the above protein phosphatase 2C gene and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene thereinto or modifying an expression control region of endogenous genes corresponding to the genes. Here, the term "significantly increased production of biomass" refers to a situation in which the total weight of each plant is statistically significantly increased compared with the same of a wild-type plant. In this case, even when some plant tissues become specifically large and the sizes of the other tissues are equivalent to those of a wild-type plant, it is concluded that the production of biomass is increased if the total weight of the entire plant is large. In addition, the term " significantly increased production of seeds" refers to a situation in which the total amount and/or total number of seeds harvested from each plant is statistically significantly increased compared with the same of a wild-type plant. Specifically, such a situation may be any of a situation in which the size of each seed (grain) is improved, a

situation in which the size of each seed (grain) is equivalent to the other but the number of seeds is improved, or a situation in which the size of each seed (grain) is improved and the number of seeds is improved.

[0114]

According to the present invention, the production of biomass and/or seeds by plants is increased. Hence, improvement in productivity can be achieved in both of the following cases: a case in which a purpose is to produce the whole plant; and a case in which a purpose is to produce some plant tissues (e.g., seeds) or components contained in plants. For example, when a purpose is to produce fats and oils contained in plant seeds, the amounts of fats and oils that can be harvested per area under cultivation can be greatly improved. Here, examples of fats and oils include, but are not particularly limited to, plant-derived fats 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. Also, the thus produced fats and oils can be broadly used for household uses or industrial uses and can be further used as raw materials for biodiesel fuel. Hence, according to the present invention, the above fats and oils for household uses or industrial uses, biodiesel fuel, and the like can be produced at low cost with the use of plants into which the above protein phosphatase 2C gene and the above glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene have been introduced or in which an expression control region of endogenous genes corresponding to the genes has been modified.

[0115]

It is industrially very important to further enhance a useful trait through a combination of a plurality of techniques for modifying a gene exhibiting the useful trait via activation of gene expression. However, it cannot be said that the determination of gene modification techniques to be combined in such a way is always easy. When many genes are subjected to technical development, many combinations may need to be evaluated. The protein phosphatase 2C genes that are used in the present invention are assumed to be

involved in signal transduction with which plant hormones and particularly gibberellic acid and abscisic acid are associated. Hence, we attempted to use a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene that is associated with photosynthesis in one combination and thought to have a weak association with the above signal transduction. This combination resulted in additive effects in increased production of biomass and/or seeds of a plant into which both genes had been introduced or in which an expression control region of the endogenous genes corresponding to the genes had been modified. These results indicate that additive effects can be expected when a gene associated with signal transduction (with which gibberellic acid or abscisic acid is associated) and a gene for which a change in the above signal transduction does not serve as a major factor for increased production of biomass and/or seeds are used in combination, as in the case of a protein phosphatase 2C gene. Examples of such a gene include AINTEGUMENTA (Proc. Natl. Acad. Sci. U.S.A., 97 942-947, (2000)), ARGOS (Plant Cell, 15, 1951-1961, (2003)), ARL (Plant J., 47, 1-9, (2006)), AVP1 (Science, 310, 121-125, (2005)), and ARF2 (Development, 133, 251-261, (2006)).

[Examples]

[0116]

The present invention will be specifically described in the following reference examples and examples. However, the examples are not intended to limit the technical scope of the present invention.

[0117]

[Reference example 1]

1. Materials and methods

1-1. Experimental materials

As experimental materials, seeds of *Arabidopsis thaliana* mutants (Activation-tag lines: Weigel T-DNA lines, 20072 lines) were used. In addition, the seeds were purchased from the Nottingham Arabidopsis Stock Centre (NASC). Regarding the seeds used as

experimental materials, Weigel, D. et al., 2000, Plant Physiol. 122, 1003-1013 can be referred to.

[0118]

1-2. Methods

1-2-1. Selection of salt-resistant mutants

Seeds of Weigel T-DNA lines were aseptically sowed on 125 mM or 150 mM NaCl-containing modified MS agar (1%) medium [vitamins in B5 medium, 10 g/l sucrose, and 8 g/L agar (for bacterial medium; Wako Pure Chemical Industries, Ltd.)] and then cultured at 22°C under 30-100 μmol/m^2/sec illumination (a cycle of 16 hours in the light/8 hours in the dark). Two to 4 weeks after sowing, salt-resistant mutant candidates were selected. In addition, regarding MS medium, see Murashige, T. et al., 1962, Physiol. Plant. 15, 473-497. Also, regarding the B5 medium, see Gamborg, O. L. et al., 1968, Experimental Cell Research 50, 151-158.

[0119]

1-2-2. DNA preparation

A site for insertion of T-DNA into the genome of the thus selected salt-resistant *Arabidopsis thaliana* line was determined by a TAIL-PCR method. First, young leaves were harvested from the cultivated *Arabidopsis thaliana* plants and then crushed under liquid nitrogen freezing. DNA was prepared using a DNA preparation kit (DNeasy Plant Mini Kit, QIAGEN) according to the standard protocols included with the kit.

[0120]

1-2-3. TAIL-PCR method and presumption of T-DNA insertion site

Three (3) types of specific primer, TL1, TL2, and TL3, were determined to be located near the left T-DNA sequence (T-DNA left border) of an activation-tagging vector (pSKI015: GenBank accession No. AF187951) used in Weigel T-DNA lines. With the use of an arbitrary primer P1 and the following PCR reaction solutions and reaction conditions, TAIL-PCR (supervisors, Isao Shimamoto and Takuji Sasaki, New Edition, Plant PCR

Experimental Protocols, 2000, pp. 83-89, Shujunsha, Tokyo, Japan; Liu, Y.G. and Whttier, R. F., 1995, Genomics 25, 674-681; Liu, Y.G. et al., Plant J., 8, 457-463, 1995) was performed, so that genomic DNA adjacent to T-DNA was amplified.

[0121]

The specific sequences of the primers TL1, TL2, TL3, and P1 are as follows.

[0122]

TL1: 5'-TGC TTT CGC CAT TAA ATA GCG ACG G-3' (SEQ ID NO: 24)

TL2: 5'-CGC TGC GGA CAT CTA CAT TTT TG-3' (SEQ ID NO: 25)

TL3: 5'-TCC CGG ACA TGA AGC CAT TTA C-3' (SEQ ID NO: 26)

P1: 5'-NGT CGA SWG ANA WGA A-3' (SEQ ID NO: 27)

In addition, in SEQ ID NO: 25, "n" represents "a," "g," "c," or "t" (location: 1 and 11), "s" represents "g" or "c" (location: 7), and "w" represents "a" or "t" (location: 8 and 13).

[0123]

The 1st PCR reaction solution composition and reaction conditions are shown in Table 1 and Table 2, respectively.

[Table 1]

Template (genomic DNA)	: 10 ng
10xPCR buffer (Takara Bio)	: 2 μl
2.5 mM dNTPs (Takara Bio)	: 1.6 μl
1st specific primer (TL1: SEQ ID NO: 24)	: 0.5 pmol
Arbitrary primer 1 (SEQ ID NO: 27)	: 100 pmol
TaKaRa Ex Taq (Takara Bio)	: 1.0 unit
Total	20 μl

[Table 2]

#1: 94°C (30 seconds)/95°C (30 seconds)

#2: 5 cycles of 94°C (30 seconds)/65°C (30 seconds)/72°C (1 minute)

#3: 1 cycle of 94°C (30 seconds)/25°C (1 minute) raised to 72°C within 3 minutes/72°C (3 minutes)

#4: 94°C (15 seconds)/65°C (30 seconds)/72°C (1 minute)

94°C (15 seconds)/68°C (30 seconds)/72°C (1 minute)

15 cycles of 94°C (15 seconds)/44°C (30 seconds)/72°C (1 minute)

#5: 72°C (3 minutes)

[0124]

The 2nd PCR reaction solution composition and reaction conditions are shown in Table 3 and Table 4, respectively.

[Table 3]

Template (50-fold dilution of the 1st PCR product)	: 1 μl
10xPCR buffer (Takara Bio)	: 2 μl
2.5 mM dNTPs (Takara Bio)	: 1.5 μl
2nd specific primer (TL2: SEQ ID NO: 25)	: 5 pmol
Arbitrary primer 1 (SEQ ID NO: 27)	: 100 pmol
TaKaRa Ex Taq (Takara Bio)	: 0.8 unit
Total	20 μl

[Table 4]

#6: 94°C (15 seconds)/64°C (30 seconds)/72°C (1 minute)

94°C (15 seconds)/64°C (30 seconds)/72°C (1 minute)

12 cycles of 94°C (15 seconds)/44°C (30 seconds)/72°C (1 minute)

#5: 72°C (5 minutes)

[0125]

The 3rd PCR reaction solution composition and reaction conditions are shown in Table 5 and Table 6, respectively.

[Table 5]

Template (50-fold dilution of the 2nd PCR product)	: 1 μl
10xPCR buffer (Takara Bio)	: 5 μl
2.5 mM dNTPs (Takara Bio)	: 0.5 μl
3rd specific primer (TL3 : SEQ ID NO: 26)	: 10 pmol
Arbitrary primer 1 (SEQ ID NO: 27)	: 100 pmol
TaKaRa Ex Taq (Takara Bio)	: 1.5 unit
Total	50 μl

[Table 6]

#7: 20 cycles of 94°C (30 seconds)/44°C (30 seconds)/72°C (1 minute)

#5: 72°C (3 minutes)

[0126]

Subsequently, the 2nd and the 3rd reaction products were subjected to agarose gel electrophoresis and then the presence or the absence of amplification and the specificity of reaction products were confirmed. Also, the 3rd amplification products were subjected to a sequencing reaction directly using a BigDye Terminator Cycle Sequencing Kit Ver. 3. 1 (Applied Biosystems) and the specific primer TL3. Thus, a nucleotide sequence was determined using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). As a result, 498-bp sequence information was obtained (SEQ ID NO: 28).

[0127]

The Arabidopsis Information Resource (TAIR: http: //www.arabidopsis.org/) was subjected to a BLAST search for the thus obtained sequence. Thus, the insertion site was found to be the gene of [AGI (Arabidopsis Genome Initiative gene code) code: At3g05630] of *Arabidopsis thaliana* chromosome 3.

[0128]

1-2-4. Prediction of activated genes

Activated genes were predicted from the sequence of a presumed open reading frame

(ORF) gene existing within a 10-Kb range near the T-DNA insertion site (At3g05630) revealed in 1-2-3.

[0129]

1-2-5. Obtainment of predicted genes

For amplification of a fragment containing the ORF region of PP2C (protein phosphatase 2C) gene (At3g05640) predicted to be activated in 1-2-4, PCR primers 5640PF1 and 5640PR1 were designed and synthesized based on the sequence information disclosed at the TAIR (<http://www.arabidopsis.org/home.html>). In addition, these primers were designed, so that a restriction enzyme site (*Bsr*G I or *Sal* I) required for introduction into expression vectors was added to the terminus of each primer.

[0130]

5640PF1(SEQ ID NO: 29):

5'-ACG CGT CGA CAT GGG ACA TTT CTC TTC CAT GTT CAA CGG-3'

5640PR1(SEQ ID NO: 30):

5'-TGT ACA TGT ACA CTA TAG AGA TGG CGA CGA CGA TGA AGA ATG G-3'

According to the method described in 1-2-2, a template DNA was prepared from wild-type *Arabidopsis thaliana* (ecotype Col-0). Phusion High-Fidelity DNA Polymerase (New England BioLabs: NEB) was used as an enzyme and the above 5640PF1 and 5640PR1 were used as primers. The relevant PCR reaction solution composition and reaction conditions are shown in Table 7 and Table 8, respectively.

[Table 7]

Template (genomic DNA)	: 60 ng
10xHF buffer (NEB)	: 5 μl
10 mM dNTPs (NEB)	: 1.0 μl
Each primer	: 20 pmol
Phusion High-Fidelity DNA Polymerase	: 1.0 unit

Total	50 μl

[Table 8]

#1: 98°C (30 seconds)

#2: 15 cycles of 98°C (10 seconds)/55°C (30 seconds)/72°C (30 seconds)

#5: 72°C (10 minutes)

[0131]

PCR amplification products were subjected to electrophoresis with 2% agarose gel (TAE buffer) and then fragments were stained with ethidium bromide. A gel containing target fragments was excised using a scalpel. Target DNA fragments were eluted and purified using GFX PCR DNA and a GEL Band Purification Kit (Amersham). Adenin was added to the thus obtained DNA fragment using an A-Addition Kit (QIAGEN). The amplified DNA to which adenine had been added was ligated to a TA-Cloning pCR2.1 vector using a TOPO TA Cloning Kit (Invitrogen) and then transformed into competent cells (*E. coli* TOP 10) included with the kit. After transformation, cells were cultured in LB medium supplemented with 50 μl/ml kanamycin and then transformants were selected. Colonies that had appeared were subjected to liquid culture in LB medium supplemented with 50 μl/ml kanamycin. Plasmid DNA was prepared from the thus obtained microorganisms using a Plasmid Mini Kit (QIAGEN). The thus obtained fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) was cloned into a vector, followed by determination of the nucleotide sequence and sequence analysis.

[0132]

1-2-6. Construction of plant expression vector

A fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) was inserted into a plant expression vector pBI121 containing an omega sequence from tobacco mosaic virus. Thus, a construct was prepared.

[0133]

First, the pCR2.1 vector, in which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) had been cloned in 1-2-5, was treated with restriction enzymes Sal I and *Bsr*G I.

[0134]

Next, similarly pBI121 containing an omega sequence was treated with restriction enzymes *Sal* I and *Bsr*G I. The products digested with these restriction enzymes were subjected to 0.8% agarose gel electrophoresis. A fragment of about 2700 bp containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) and pBI121 containing the omega sequence were each fractioned and purified from the gel using GFX PCR DNA and a GEL Band Purification Kit (Amersham).

[0135]

For introduction of a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) using a pBI121 fragment containing the omega sequence as a vector, the vector and the insert were mixed at a ratio of 1 : 10, followed by an overnight ligation reaction at 16°C using an equivalent amount of a TaKaRa Ligation kit ver. 2 (Takara Bio Inc.).

[0136]

The total amount of the reaction solution was added to 100 μl of competent cells (*E. coli* strain DH5α, TOYOBO), so that transformation was performed according to protocols included with the kit. Cells were applied to LB agar medium containing 50 μg/ml kanamycin and then cultured overnight. Colonies that had appeared were subjected to liquid culture in LB medium supplemented with 50 μg/ml kanamycin. Plasmid DNA was prepared from the thus obtained microorganisms using a Plasmid Mini Kit (QIAGEN).

[0137]

The thus obtained fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) was subcloned into an expression vector, followed by determination of the nucleotide sequence and sequence analysis.

[0138]

1-2-7. Gene introduction into *Arabidopsis thaliana* using Agrobacterium method

The plant expression vector constructed in 1-2-6 was introduced into *Agrobacterium tumefaciens* C58C1 strain by electroporation (Plant Molecular Biology Mannal, Second Edition, B. G. Stanton and A. S. Robbert, Kluwer Acdemic Publishers 1994). Subsequently, *Agrobacterium tumefaciens* in which the plant expression vector had been introduced was introduced into wild-type *Arabidopsis thaliana* (ecotype Col-0) by an infiltration method described by Clough et al. (Steven J. Clough and Andrew F. Bent, 1998, The Plant Journal 16, 735-743).

[0139]

Transformants were selected using kanamycin-containing medium. T1 generation plants were produced by self-pollination from the transformants, so that T2 seeds were obtained.

[0140]

1-2-8. Confirmation of the phenotype of transformant

T2 seeds produced in 1-2-7 were aseptically sowed and then the resulting plants were transplanted into pots (each with a diameter of 50 mm) containing vermiculite mixed soil. As control plants for comparison, Arabidopsis plants that had not undergone recombination were transplanted. They were cultivated under conditions of 22°C and 16 hours in the light/8 hours in the dark, and with a light intensity ranging from about 30 to 45 $\mu mol/m^{-2}/s^{-1}$, for a total of 11 weeks after transplantation. After cultivation, above-ground parts of the plants were placed in paper bags and dried under conditions of 22°C and humidity of 60% for 2 weeks. The total amounts of biomass and seeds were weighed using an electronic balance.

[0141]

1-3. Results

Regarding the results of 1-2-8, Fig. 5 shows a photo of the above-ground parts of wild-type plants and transformed plants into which a fragment containing ORF of the PP2C

(protein phosphatase 2C) gene (At3g05640) had been introduced. Also, Fig. 6 and Fig. 7 show the results of measuring the total amounts of biomass and seeds of the above-ground parts of the plants.

[0142]

As shown in Figs. 5, 6, and 7, it was revealed that in the case of transformed plants into which the fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) had been introduced, the total amounts of biomass of the above-ground parts were much higher (about 1.9 to 2.1 times) than the amounts of the same in the cases of wild-type plants. In addition, the amounts of seeds were much greater (by about 1.7 to 1.8 times) than the same in the cases of wild-type plants.

[0143]

[Example 1]

In Example 1, transformed plants were produced by introducing a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene (hereinafter, FBA1 gene) into transformed plants into which the PP2C (protein phosphatase 2C) gene (At3g05640) had been introduced.

[0144]

2. Materials and methods

2-1. Experimental materials

As experimental materials, the seeds of T3 (or later) plants of transformed plants into which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) prepared in 1-2-7 had been introduced, were used. Wild-type *Arabidopsis thaliana* (ecotype Col-0) was used.

[0145]

The plants were transplanted into square plastic pots (6.5 x 6.5 x 5 cm) containing, from the bottom, vermiculite (Asahi Kogyo), Kureha culture soil (Kureha gardening culture soil, Kureha Corporation), and vermiculite at a ratio of 2 : 2 : 1 to form 3 layers. They were

grown under long-day conditions of 22°C (growth temperature) and 16 hours in the light/8 hours in the dark.

[0146]

2-2. Methods

2-2-1. Obtainment of FBA1 gene (At2g01140)

Total RNA was isolated from 4-week-old *Arabidopsis thaliana* (wild-type Columbia (Col-0)), RT-PCR (amount of template RNA; 5.0 μg) was performed using a Prost arfirststrand RT-PCR kit (Stratagene), and thus cDNA was constructed.

[0147]

PCR was performed using the following specific primers that had been designed based on the cDNA sequence (SEQ ID NO: 31) of the FBA1 gene (At2g01140), so as to amplify the full-length cDNA as two fragments. The thus amplified fragments were each TA-cloned into a pGEM-T vector (Promega).

[0148]

1F-1:5'-GGATCCTATGGCGTCTGCTAG-3'(SEQ ID NO: 44)

1R-1:5'-ATCTGCAACGGTCTCGGGAGA-3'(SEQ ID NO: 45)

1F-2:5'-GTGTGGTCCGAGGTGTTCTTCT-3'(SEQ ID NO: 46)

1R-2:5'-GAGCTCGAGTAGGTGTAACCCTTG-3'(SEQ ID NO: 47)

Two fragments were fused at the *Bst*p I site, and thus a vector (pGEM-FBA1) containing the full-length cDNA was constructed. For production of transformed plants, pGEM-FBAl was treated with restriction enzymes *Bam*H I and *Sac* I and then the resulting fragment was introduced into a pBI121 vector.

[0149]

2-2-2. Construction of plant expression vector

The fragment containing the FBA1 gene (At2g01140) obtained in 2-2-1 was inserted into a pMAT137-HM plant expression vector (Matsuoka K. and Nakamura K., 1991, Proc. Natl. Acad. Sci. U.S.A. 88, 834-838), so that a construct was prepared.

[0150]

First, the fragment containing the FBA1 gene and a NOS terminator incorporated into the pBI121 vector was excised with *Xba* I and *Eco*R I and then incorporated into a pBluscriptII (SK+) vector (Stratagene) that had been treated with *Xba* I and *Eco*R I. Subsequently, the fragment containing the FBA1 gene and the NOS terminator was excised with *Xba* I and *Kpn* I and then incorporated into a pMAT137-Hm vector that had been treated with *Xba* I and *Kpn* I.

[0151]

2-2-3. Gene introduction into *Arabidopsis thaliana* using Agrobacterium method

The pMAT137-Hm plant expression vector constructed in 2-2-2 was introduced into the *Agrobacterium tumefaciens* C58C1 strain by electroporation (Plant Molecular Biology Mannal, Second Edition, B. G. Stanton and A. S. Robbert, Kluwer Acdemic Publishers 1994). Subsequently, *Agrobacterium tumefaciens* into which the plant expression vector had been introduced was introduced into wild-type *Arabidopsis thaliana* (ecotype Col-0) by an infiltration method described by Clough et al. (Steven J. Clough and Andrew F. Bent, 1998, The Plant Journal 16, 735-743).

[0152]

Transformants were selected using hygromycin-containing medium. T1 generation plants were produced by self-pollination from the transformants, so that T2 seeds were obtained.

[0153]

2-2-4. Confirmation of the phenotype of transformant

T2 seeds produced in 2-2-3 were sowed into square plastic pots (6.5 x 6.5 x 5 cm) containing, from the bottom, vermiculite (Asahi Kogyo), Kureha culture soil (Kureha gardening culture soil, Kureha Corporation), and vermiculite at a ratio of 2: 2 : 1 to form 3 layers. They were grown under long-day conditions of 22°C (growth temperature) and 16 hours in the light/8 hours in the dark. As control plants, transformed plants produced with

the introduction of the PP2C gene prepared in Reference example 1 (1-2-7) were used. They were cultivated under conditions of 22°C, 16 hours in the light/8 hours in the dark, and a light intensity ranging from about 70 to 100 $\mu mol/m^{-2}/s^{-1}$, for a total of 11 weeks after transplantation. After cultivation, above-ground parts of the plants were placed in paper bags and dried under conditions of 22°C and humidity of 60% for 2 weeks. The total amounts of biomass were weighed using an electronic balance.

[0154]

2-3. Results

Fig. 8 is a photo showing the results of 2-2-4 above and specifically the above-ground parts of wild-type plants, transformed plants produced with the introduction of a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640), and transformed plants produced by introducing the FBA1 gene (At2g01140) into the above-mentioned transformed plants (into which a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640) had been introduced). Fig. 9 shows the results of measuring the total amounts of biomass of the above-ground parts. In addition, each amount of biomass shown in Fig. 9 is the average value for 6 pots each containing 3 individual plants.

[0155]

As revealed in Fig. 8, in the case of transformed plants produced with the introduction of the FBA1 gene (At2g01140) into transformed plants that had been produced with the introduction of the fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640), the sizes of above-ground parts had improved over those of wild-type plants and the transformed plants produced with the introduction of the fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640).

[0156]

Also, as revealed in Fig. 9, in the case of the transformed plants produced by introducing the FBA1 gene (At2g01140) into the transformed plants that had been produced

with the introduction of the fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640), the total amount of biomass of the above-ground parts had improved by about 8% to 14% compared with the transformed plants produced with the introduction of a fragment containing ORF of the PP2C (protein phosphatase 2C) gene (At3g05640).

[0157]

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

[Sequence Listing]

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CLAIMS

1. A plant, into which a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase are introduced, or an expression control region of endogenous genes corresponding to the genes is modified.

2. The plant according to claim 1, wherein the gene encoding protein phosphatase 2C is at least one gene selected from the group consisting of At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270, or a gene functionally equivalent to the gene.

3. The plant according to claim 1, wherein the gene encoding protein phosphatase 2C encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 5;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5 and having protein phosphatase 2C activity; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 4 and has protein phosphatase 2C activity.

4. The plant according to claim 2, wherein the functionally equivalent gene is a protein phosphatase 2C gene from an organism other than *Arabidopsis thaliana*.

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5. The plant according to any preceding claim, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase is an At2g01140 gene or a gene functionally equivalent to the gene.

6. The plant according to any one of claims 1 to 4, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 32;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 32 and exhibiting fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 31 and exhibits fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione.

7. The plant according to claim 5, wherein the functionally equivalent gene is a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene from an organism other than *Arabidopsis thaliana*.

8. A method for increasing the production of biomass and/or seeds, comprising the introduction of a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase, or the modification of an expression control region of endogenous genes corresponding to the genes.

9. The method according to claim 8, wherein the gene encoding protein phosphatase 2C is at least one type of gene selected from the group consisting of At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270, or a gene functionally equivalent to the gene.

10. The method according to claim 8, wherein the gene encoding protein phosphatase 2C encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 5;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5 and having protein phosphatase 2C activity; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 4 and has protein phosphatase 2C activity.

11. The method according to any one of claims 8 to 10, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase is an At2g01140 gene or a gene functionally equivalent to the gene.

12. The method according to any one of claims 8 to 10, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 32;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with

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respect to the amino acid sequence shown in SEQ ID NO: 32 and exhibiting fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 31 and exhibits fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione.

13. A method for producing a plant, comprising the steps of:

preparing a transformed plant by introducing a gene encoding protein phosphatase 2C having 3 consensus sequences comprising the amino acid sequences shown in SEQ ID NOS: 1-3 from the N-terminal side in such order and a gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase, or modifying an expression control region of endogenous genes corresponding to the genes; and

measuring the amount of biomass and/or seeds of a progeny plant of the transformed plant and then selecting a line in which the amount of biomass and/or seeds is significantly improved.

14. The production method according to claim 13, wherein the gene encoding protein phosphatase 2C is at least one type of gene selected from the group consisting of At1g03590-AtPP2C6-6, At1g16220, At1g79630, At5g01700, At3g02750, At5g36250, At5g26010, At4g32950, At3g16800, At3g05640, At5g27930-AtPP2C6-7, At2g20050, and At3g06270 or a gene functionally equivalent to the gene.

15. The production method according to claim 13, wherein the gene encoding protein phosphatase 2C encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 5;

(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5 and having protein phosphatase 2C activity; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 4 and has protein phosphatase 2C activity.

16. The production method according to claim 14, wherein the functionally equivalent gene is a protein phosphatase 2C gene from an organism other than *Arabidopsis thaliana*.

17. The production method according to any one of claims 13 to 16, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase is an At2g01140 gene or a gene functionally equivalent to the gene.

18. The production method according to any one of claims 13 to 16, wherein the gene encoding glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase encodes any one of the following proteins (a) to (c):

(a) a protein comprising the amino acid sequence shown in SEQ ID NO: 32;

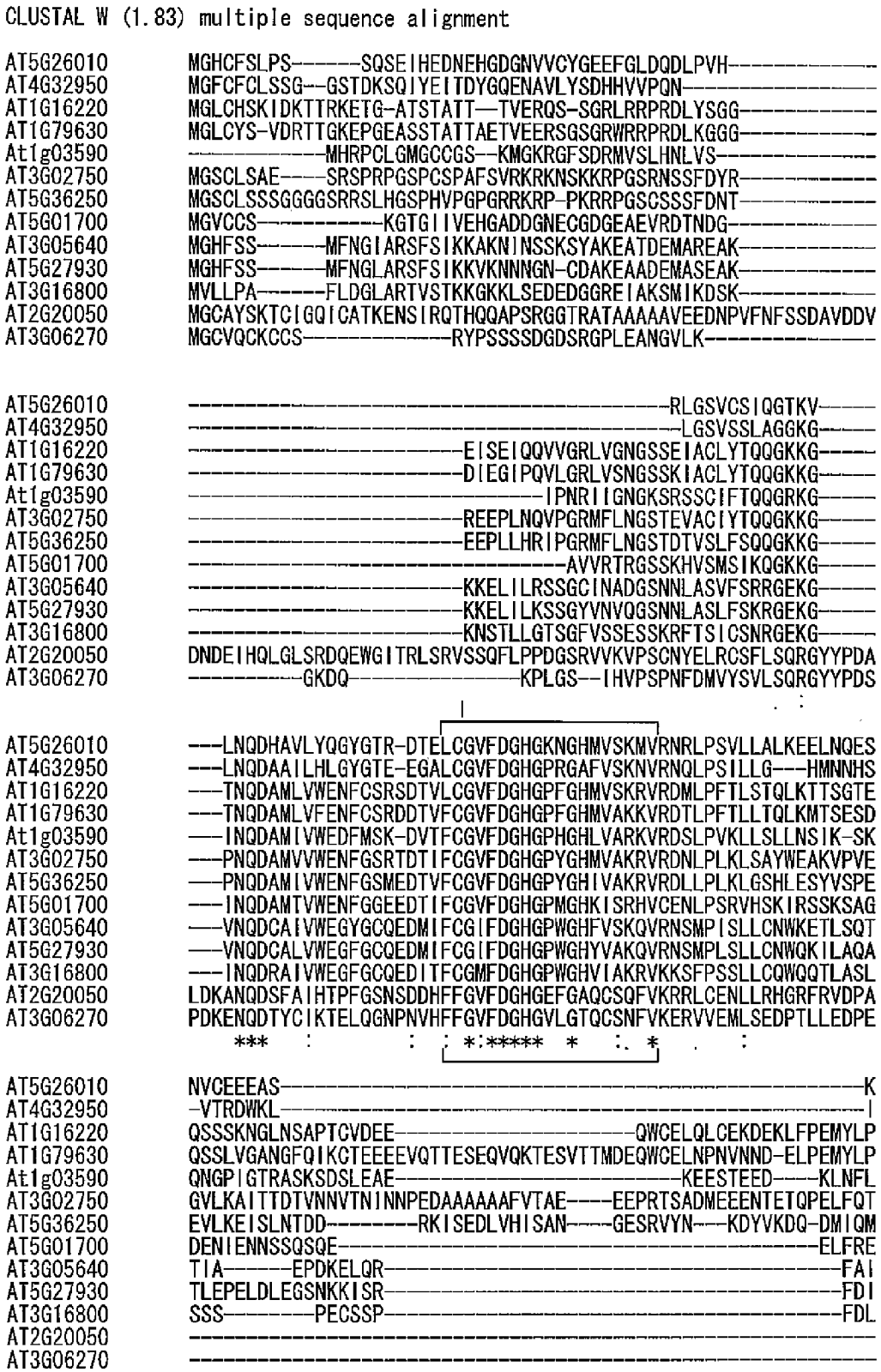
(b) a protein comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in SEQ ID NO: 32 and exhibiting fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione; and

(c) a protein that is encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence complementary to the

nucleotide sequence shown in SEQ ID NO: 31 and exhibits fructose 1,6-bisphosphate aldolase activity as a result of binding of glutathione.

19. The production method according to claim 17, wherein the functionally equivalent gene is a glutathione-binding plastid-type fructose 1,6-bisphosphate aldolase gene from an organism other than *Arabidopsis thaliana*.

[図1-1]


CLUSTAL W (1.83) multiple sequence alignment
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AT1G79630
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AT5G27930
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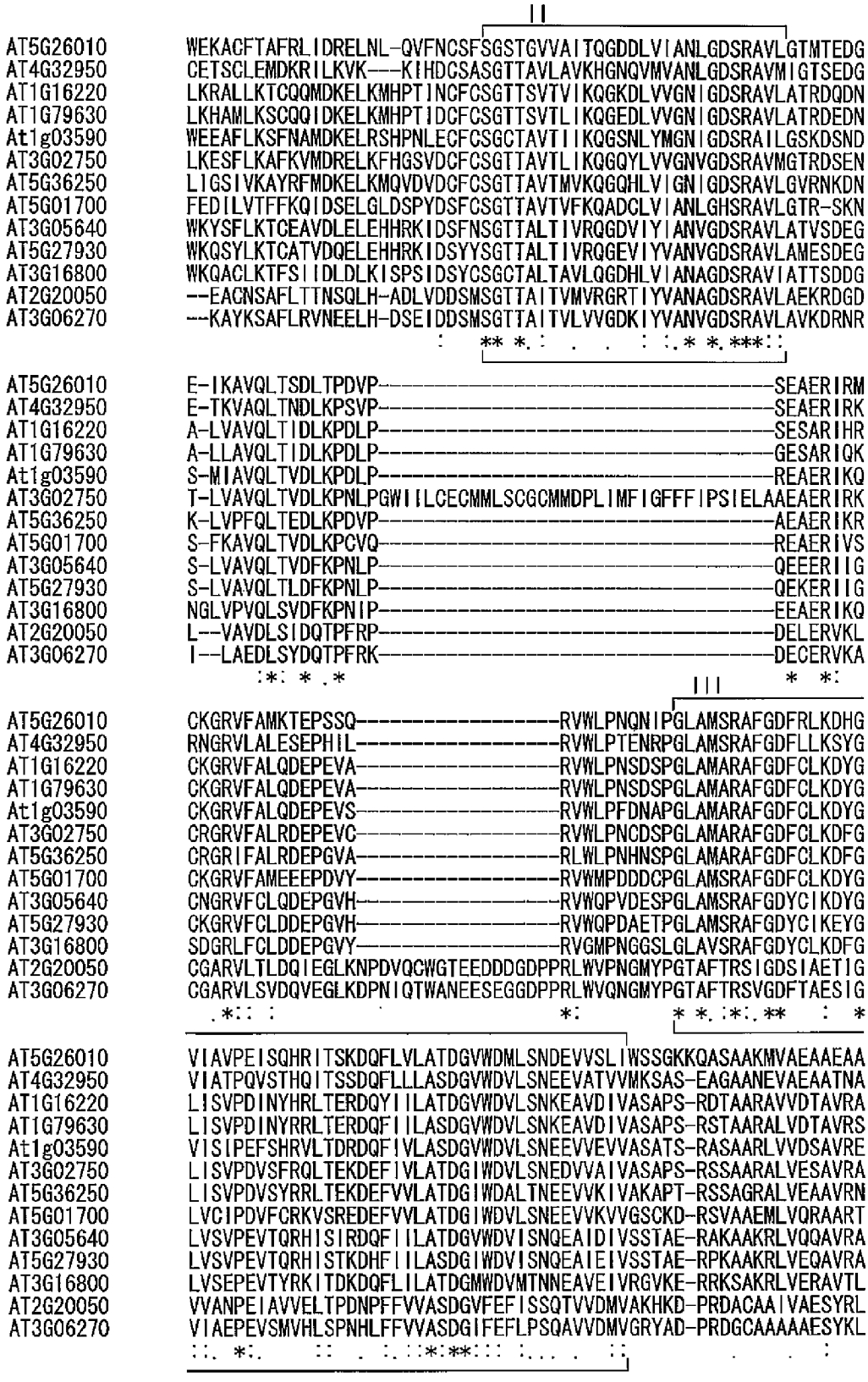
AT2G20050
AT3G06270

[図1-2]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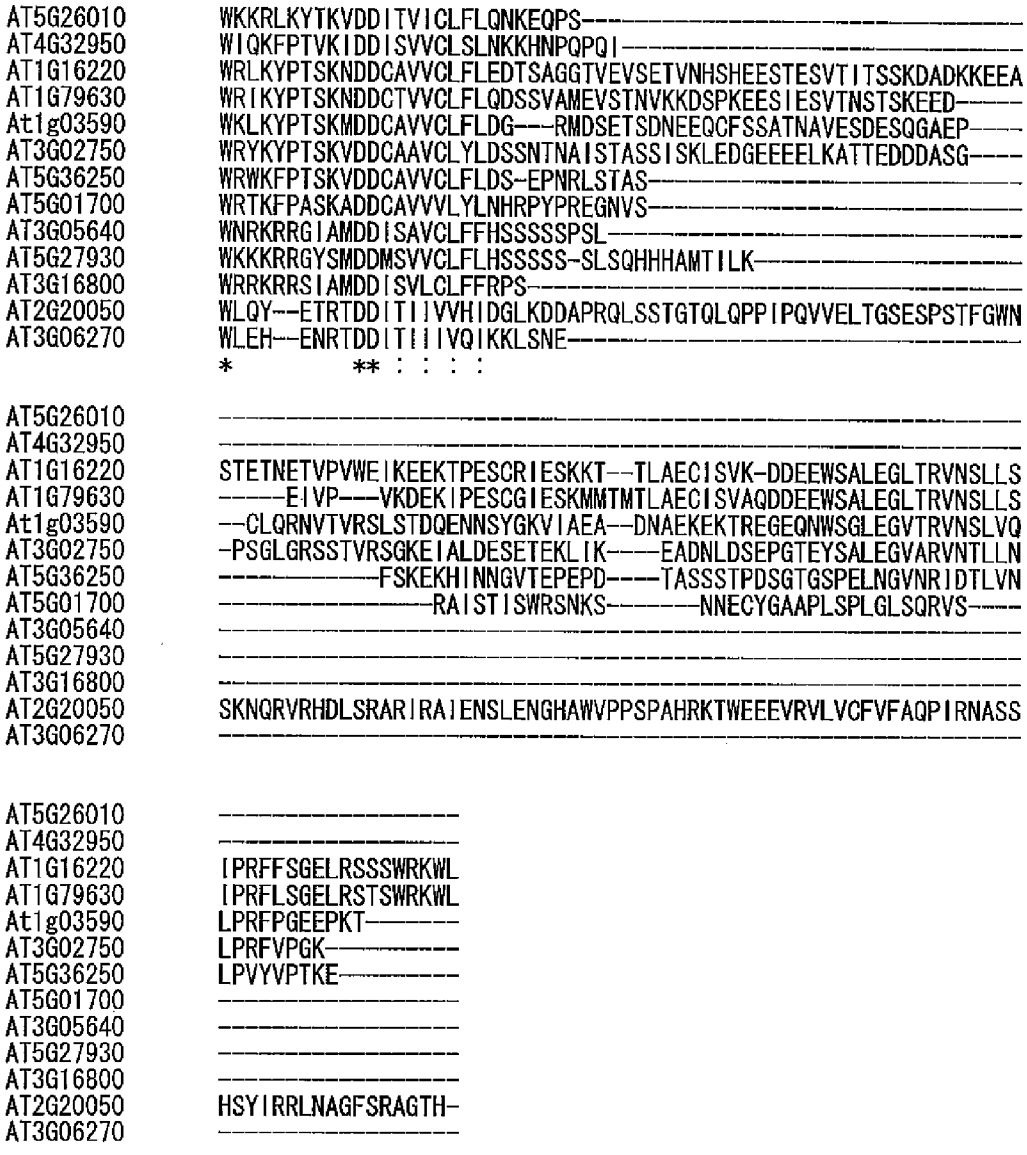
[図1-3]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AT5G36250 WRWKFPTSKVDDCAVVCLFLDS-EPNRLSTAS----------
AT5G01700 WRTKFPASKADDCAVVVLYLNHRPYPREGNVS----------
AT3G05640 WNRKRRGIAMDDISAVCLFFHSSSSSPSL----------
AT5G27930 WKKKRRGYSMDDMSVVCLFLHSSSSS-SLSQHHHAMTILK----------
AT3G16800 WRRKRRSIAMDDISVLCLFFRPS----------
AT2G20050 WLQY--ETRTDDITIIVVHIDGLKDDAPRQLSSTGTQLQPPIPQVVELTGSESPSTFGWN
AT3G06270 WLEH--ENRTDDITIIIVQIKKLSNE----------
* ** : : : :
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AT4G32950 ----------
AT1G16220 STETNETVPVWEIKEEKTPESCRIESKKT--TLAECISVK-DDEEWSALEGLTRVNSLLS
AT1G79630 -----EIVP---VKDEKIPESCGIESKMMTMTLAECISVAQDDEEWSALEGLTRVNSLLS
At1g03590 --CLQRNVTVRSLSTDQENNSYGKVIAEA--DNAEKEKTREGEQNWSGLEGVTRVNSLVQ
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AT5G01700 ----------RAISTISWRSNKS-------NNECYGAAPLSPLGLSQRVS-----
AT3G05640 ----------
AT5G27930 ----------
AT3G16800 ----------
AT2G20050 SKNQRVRHDLSRARIRAIENSLENGHAWVPPSPAHRKTWEEEVRVLVCFVFAQPIRNASS
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AT4G32950 ----------
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AT5G01700 ----------
AT3G05640 ----------
AT5G27930 ----------
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AT3G06270 ----------

[図2-1]

CLUSTAL W (1.83) multiple sequence alignment

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AT3G02750       MGSCLSAE----SRSPRPGSPCSPAFSVRKRKNSKKRPGSRNSSFDYRREEPLNQVPGRM
AT5G36250       MGSCLSSSGGGGSRRSLHGSPHVPGPGRRKRP-PKRRPGSCSSSFDNTEEPLLHRIPGRM
AT5G26010       MGHCFSLPS-----SQSEIHEDNEHGDG-NVVCYGEEFGLDQDLPVH----------
AT4G32950       MGFCFCLSSGGSTDKSQIYEITDYGQE-NAVLYSDHHVVPQN----------
AT5G01700       MGVCCSKGTG-IIVEHGADDGNECGDGEAEVRDTNDGAVVRTRGSS----------
AT3G05640       MGHFSSMFNGIARSFSIKKAKNINSSKSYAKEATDEMAREAKKKELILR-------SSGCI
AT5G27930       MGHFSSMFNGLARSFSIKKVKNNNGN-CDAKEAADEMASEAKKKELILK-------SSGYV
AT3G16800       MVLLPAFLDGLARTVSTKKGKKLSEDEDGGREIAKSMIKDSKKNSTLLG-------TSGFV
                                                          |
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AT3G02750       FLNGSTEVACIYTQQGKKGPNQDAMVVWENFGSRTDTIFCGVFDGHGPYGHMVAKRVRDN
AT5G36250       FLNGSTDTVSLFSQQGKKGPNQDAMIVWENFGSMEDTVFCGVFDGHGPYGHIVAKRVRDL
AT5G26010       -------RLGSVCSIQGTKVLNQDHAVLYQGYG-TRDTELCGVFDGHGKNGHMVSKMVRNR
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AT3G16800       SSESSKRFTSICSNRGEKGINQDRAIVWEGFGCQEDITFCGMFDGHGPWGHVIAKRVKKS
                         .:    * *  ***   :  .:    :  :**:***** *  ::: * .

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AT3G02750       LPLKLSAYWEAKVPVEGVLKAITTDTVNNVTNINNPEDAAAAAAFVTAEEEPRTSADMEE
AT5G36250       LPLKLGSHLESYVSPEEVLKEISLNTDD--------RKISEDLVHISANGESRVYN---K
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AT4G32950       LPSILLG----------HMNNHS-VTR-------------------------------
AT5G01700       LPSRVHSKIRSSKSAGDENIENNSSQSQE-----------------------------
AT3G05640       MPISLLCNWK--------ETLSQTTIA-------------------------------
AT5G27930       MPLSLLCNWQ--------KILAQATLEPE-----------------------------
AT3G16800       FPSSLLCQWQ--------QTLASLSSS-------------------------------
                :*  :
                                                          ||
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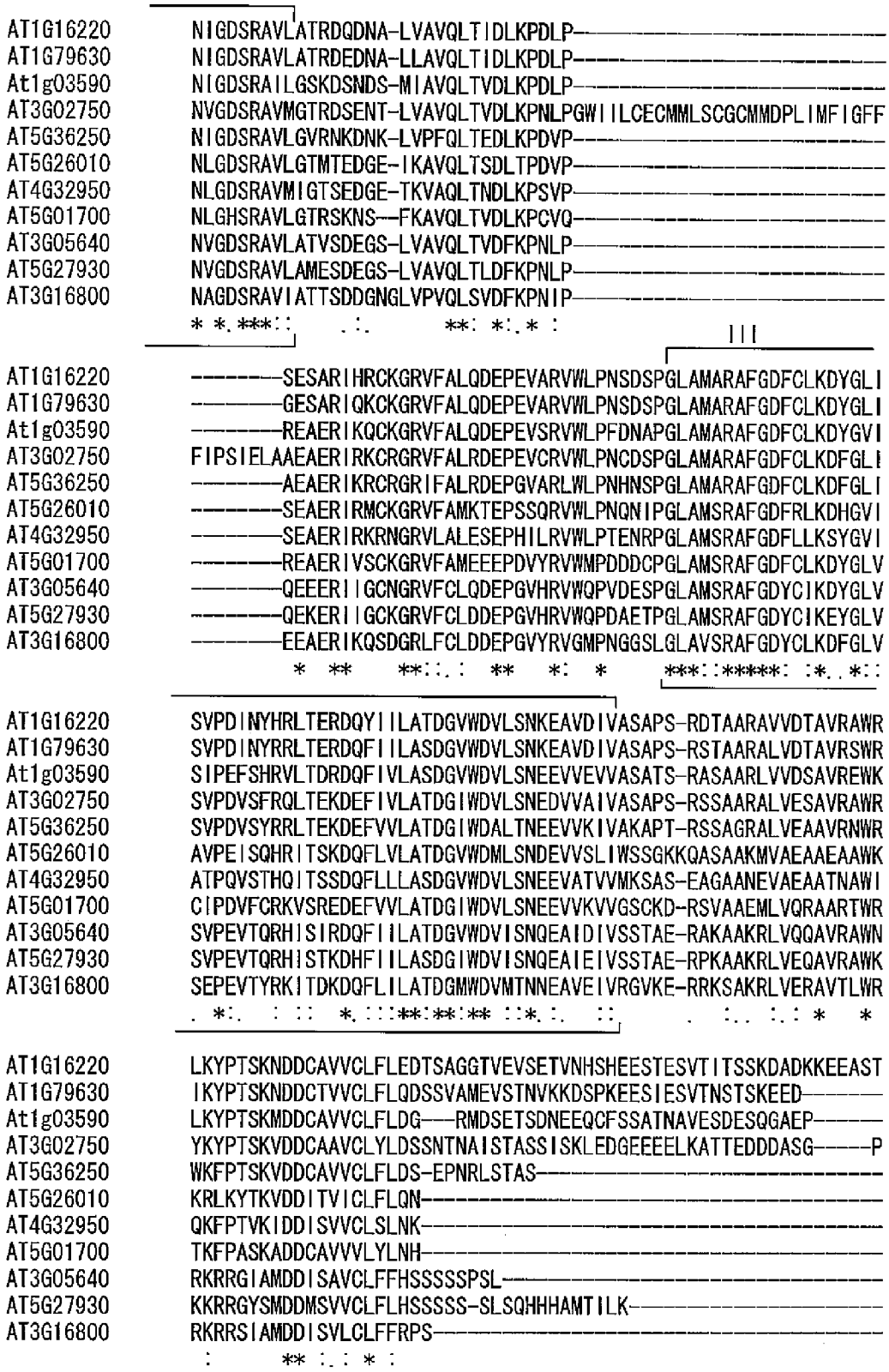
[図2-2]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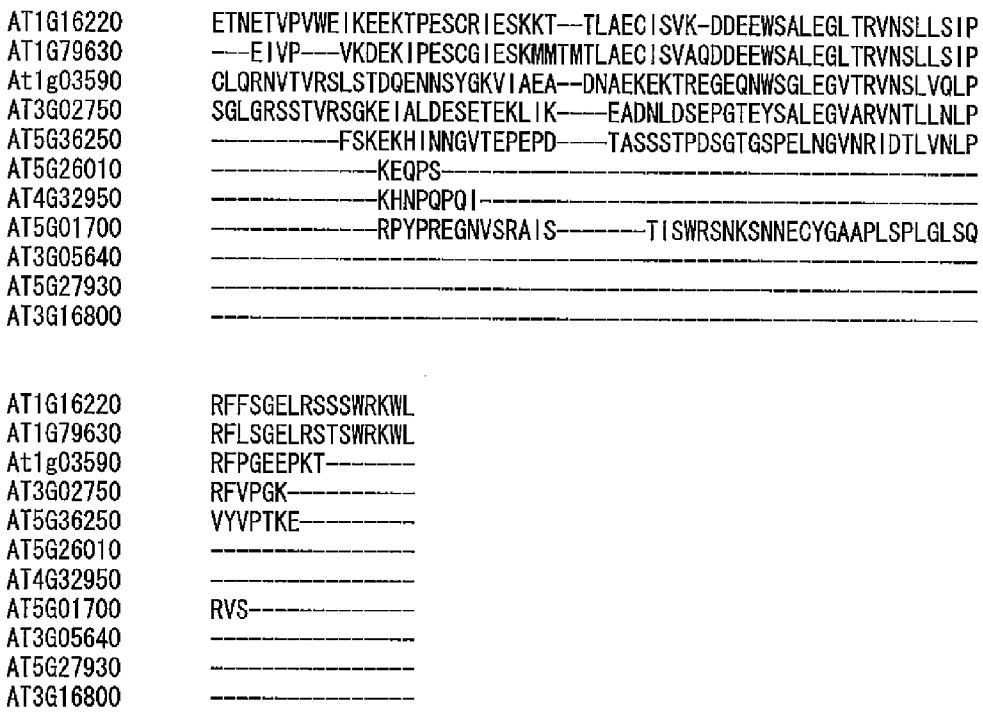
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[図3-1]

CLUSTAL W (1.83) multiple sequence alignment

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CAO42215          --MASVTFAKFNASSSQWIGQQS-FSQRQGSS-ARFPARRVSVPIRAGSYSEELVQTAKT
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ABK25226          -MAASVLKAGVRLGSSQWTGQSLTQNIDSHKQ----PKAQRVSMPIRAGSYAEELVQTAKT
ABK24568          -MAASVIKAGVRLGSSQWTGQSLTQNIDSHKQ----PKAQRVSMPIRAGSYAEELVQTAKT
ACG47464          MAMATAKLNSP--ATSLVAGGLTRRSAPARCT--------TVIRAAAGSYSDELISTAKS
ACG47669          MAMATAKLNSP--ATSLVAGGRTRRSAPARCT--------TVIRAAAGSYSDELISTAKS
Os01g0118000      MAMLTAKLTSPPAATTWLPGG-GRRSAPPRRA--------TVIRAAAVSYADELVSTAKS
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ABK94899          IASPGRGILAIDESNATCGKRLASIGLDNTETNRQAYRQLLLTTPSLGEYISGAILFEET
EEE88847          IASPGRGILAIDESNATCGKRLASIGLDNTETNRQAYRQLLLTTPGLGEYISGAILFEET
EEF36097          VASPGRGILAIDESNATCGKRLASIGLDNNETNRQAYRQLLLTTPGLGEYISGAILFEET
CAO42215          VASPGRGILAIDESNATCGKRLASIGLDNTEPNRQAYRQLLLTTPGLGEYISGAILFEET
At2g01140         IASPGRGILAIDESNATCGKRLASIGLDNTEDNRQAYRQLLLTTPGLGDYISGSILFEET
ABK24286          VASPGRGILAIDESNATCGKRLASIGLENNETNRQAYRQLLLTTPGLGEYISGSILFEET
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EEE88847          LYQSTTDGRKFVDCLRDENIVPGIKVDKGLVPLPGSNNESWCQGLDGLASRSAEYYKQGA
EEF36097          LYQSTTDGKKFVDCLRDQNIVPGIKVDKGLVPLPGSNNESWCQGLDGLASRSAEYYKQGA
CAO42215          LYQSTTDGKKFVDCLREKKIVPGIKVDKGLVPLPGSNNESWCQGLDGLASRSAEYYKQGA
At2g01140         LYQSTKDGKTFVDCLRDANIVPGIKVDKGLSPLAGSNEESWCQGLDGLASRSAEYYKQGA
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ABK25226          LYQSTTDGRKFVDCLREQNIMPGIKVDKGLVPLPGSNNESWCQGLDGLASRSAEYYKQGA
ABK24568          LYQSTTDGRKFVDCLREQNIMPGIKVDKGLVPLPGSNNESWCQGLDGLASRSAEYYKQGA
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[図3-2]

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EEE88847        RFAKWRTVVSIPCGPSALAVKEAAWGLARYAAISQDNGLVPIVEPEILLDGDHPIDRTLE
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At2g01140       RFAKWRTVVSVPCGPSALAVKEAAWGLARYAAISQDNGLVPIVEPEILLDGDHPIERTLE
ABK24286        RFAKWRTVVSIPNGPSDLAVKEAAWGLARYAAISQDNGLVPIVEPEILLDGDHSIDRTLE
ABK25226        RFAKWRTVVSIPNGPSDLAVKEAAWGLARYAAISQDNGLVPIVEPEILLDGDHSIDRTLE
ABK24568        RFAKWRTVVSIPNGPSDLAVKEAAWGLARYAAISQDNGLVPIVEPEILLDGDHSIDRTLE
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ACG47669        RFAKWRTVVSIPCGPSALAVKEAAWGLARYAAIAQDNGLVPIVEPEILLDGDHGIEGALE
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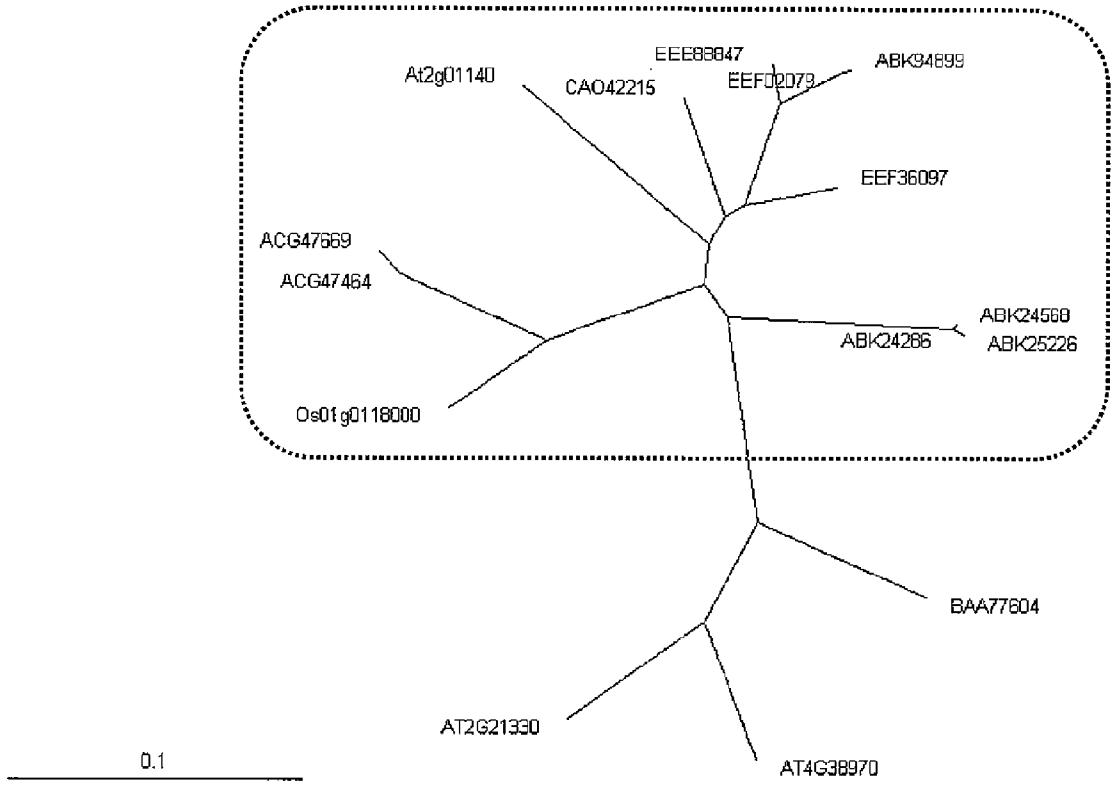
EEE88847        VAEKVWAEVFYYLAENNVVFEGILLKPSMVTPGAEHKEKASPDTIAKYTLTMLKRRVPPA
EEF36097        VAEKVWAEVFFYLAENNVVFEGILLKPSMVTPGAEHKEKASPDTIAKYTLTMLRRRVPPA
CA042215        VAEKVWSEVFFYLAQNNVLFEGILLKPSMVTPGAEHKEKASPETIAKYTLTMLRRRVPPA
At2g01140       VAEKVWSEVFFYLAQNNVMFEGILLKPSMVTPGAEHKNKASPETVADFTLTMLKRRVPPA
ABK24286        VAEKVWAEVFFYLAENNVFFEGILLKPSMVTPGAEHKEKATPQQVADYTLKMLKRRVPPA
ABK25226        VAEKVWAEVFFYLAENNVFFEGILLKPSMVTPGAEHKEKATPQQVADYTLKMLKRRVPPA
ABK24568        VAEKVWAEVFFYLAENNVLFEGILLKPSMVTPGAEHKEKATPQQVADYTLKMLKRRVPPA
ACG47464        VAEKVWSEVFFYLAENNVLFEGILLKPSMVTPGAEHKEKASPEAIAKYTLTMLRRRVPPA
ACG47669        VAEKVWSEVFFYLAENNVLFEGILLKPSMVTPGAEHKEKASPEAIAKYTLTMLRRRVPPA
Os01g0118000    VAEKVWSEVFFYLAQNNVLFEGILLKPSMVTPGAEHKQKATPEAIAKHTLTMLRRRVPPA
                ******: **:***:***.**********:**:. : :*..**.**:******

EEF02079        VPGIMFLSGGQSEVQATLNLNAMNQSPNPWHVSFSYARALQNTVLKTWQGRPDNVEAAQK
ABK94899        VPGIMFLSGGQSEVQATLNLNAMNQSPNPWHVSFSYARALQNTVLKTWQGRPDNVEAAQK
EEE88847        VPGIMFLSGGQSEVQATLNLNAMNQSPNPWHVSFSYARALQNTVLKTWQGRPENVEAAQK
EEF36097        VPGIMFLSGGQSEVEATLNLNAINQSPNPWHVSFSYARALQNSVLKTWQGHPENVEAAQK
CA042215        VPGIMFLSGGQSEVEATLNLNAMNQSPNPWHVSFSYARALQNTVLKTWQGHPENVEAAQK
At2g01140       VPGIMFLSGGQSEAEATLNLNAMNQSPNPWHVSFSYARALQNSVLRTWQGKPEKIEASQK
ABK24286        VPGIMFLSGGQSEVEATLNLNAMNQSPNPWHVSFSYARALQNTSLKTWKGLPENIEAAQR
ABK25226        VPGIMFLSGGQYEVEATLNLNAMNQSPNPWHVSFSYARALQNTSLKTWKGLPENIEAAQR
ABK24568        VPGIMFLSGGQSEVEATLNLNAMNQSPNPWHVSFSYARALQNTSLKTWKGLPENIEAAQR
ACG47464        VPGIMFLSGGQSEVEATLNLNAMNQSLNPWHVSFSYVRALQNSVLKTWQGRPENVEAAQK
ACG47669        VPGIMFLSGGQSEVEATLNLNAMNQSLNPWHVSFSYVRALQNSVLKTWQGRPENVEAAQK
Os01g0118000    VPGIMFLSGGQSEVEATLNLNAMNQEPNPWHVSFSYARALQNSVLKTWQGRPENVEAAQK
                *********** *.:*******:**. *********.*****: *:**:* *:::**:*:

EEF02079        SLLVRAKANSLAQLGRYSAEGESEEATKGMFVKGYTY---------
ABK94899        SLLVRAKANSLAQLGRYSAEGESEEATKGMFVKGYTY---------
EEE88847        SLLVRAKANSLAQLGRYSAEGESEEAKKGMFVKGYTY---------
EEF36097        ALLVRAKANSLAQLGKYSAEGENEEAKKGMFVKGYTY---------
CA042215        SLLVRAKANSLAQLGKYSAEGESEEAKKGMFVQGYTY---------
At2g01140       ALLVRAKANSLAQLGKYSAEGENEDAKKGMFVKGYTY---------
ABK24286        ALLIRAKANSLAQLGRYSAEGESEESKKGMFVKGYTY---------
ABK25226        ALLIRAKANSLAQLGRYSAEGESEESKKGMFVKGYTY---------
ABK24568        ALLIRAKANSLAQLGRYSAEGESEESKKGMFVKGYTY---------
ACG47464        ALLVRAKANSLAQLGRYTGEGESDDAKKGMFQKGYTY---------
ACG47669        ALLVRAKANSLAQLGRYTGEGESDDAKKGMFQKGYTLMCQRDVSMT
Os01g0118000    ALLVRAKANSLAQLGRYTGEGESDEAKKGMFQKGYTY---------
                :**:***********:*:.***.:::.**** :***
```

[図4]


At2g01140
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ABK94899
EEF36097
ACG47669
ACG47464
ABK24566
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ABK25226
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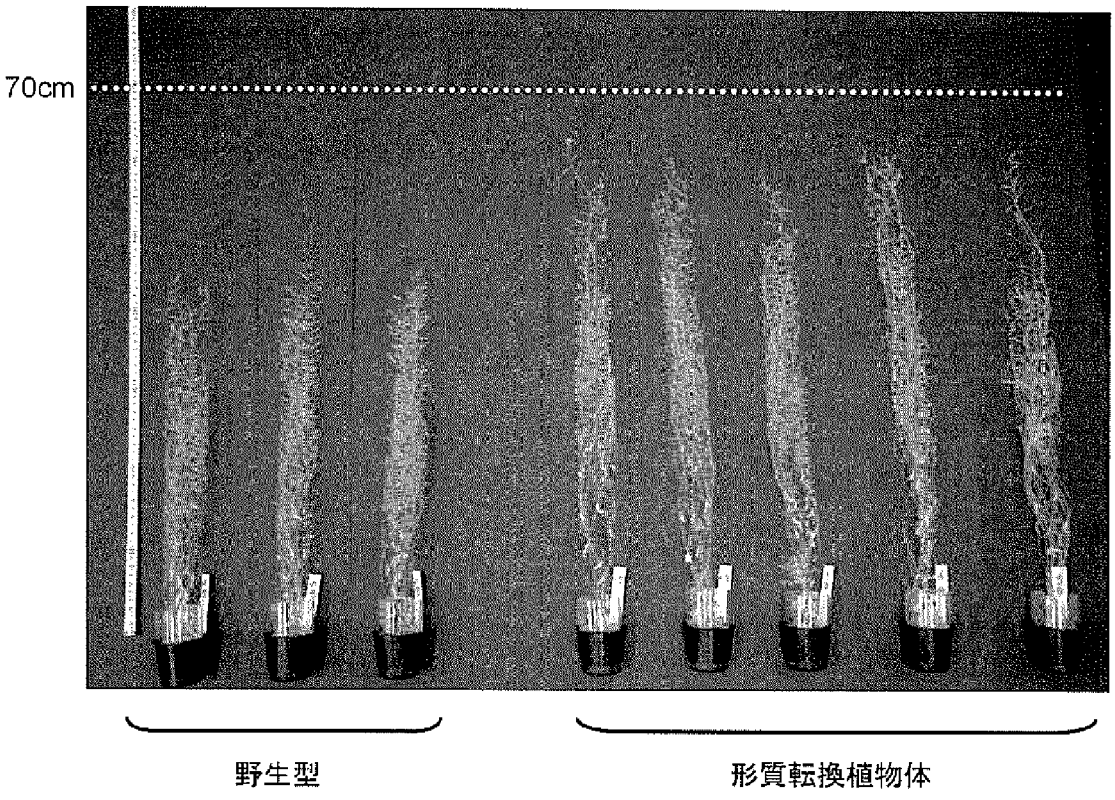
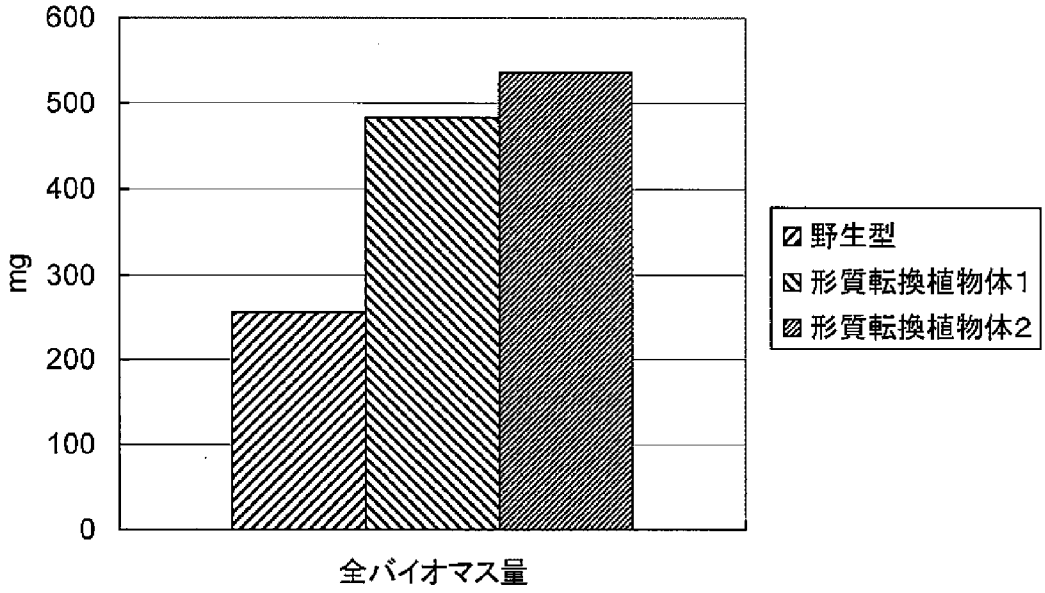
BAA77604
AT2G21330
AT4G38970
0.1

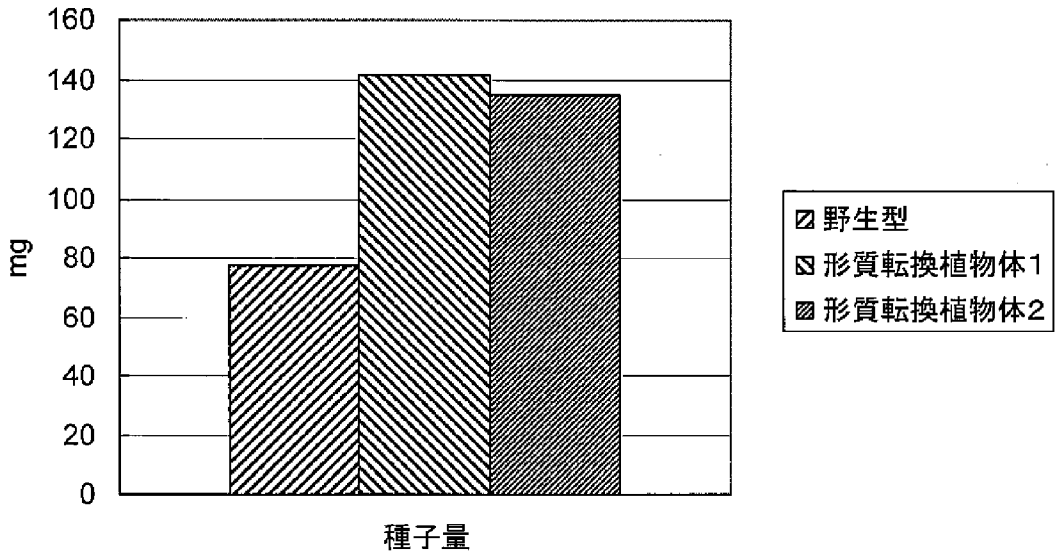
[図5]


70cm
野生型
形質転換植物体

[図6]


600
500
400
300
200
100
0
mg
野生型
形質転換植物体1
形質転換植物体2
全バイオマス量

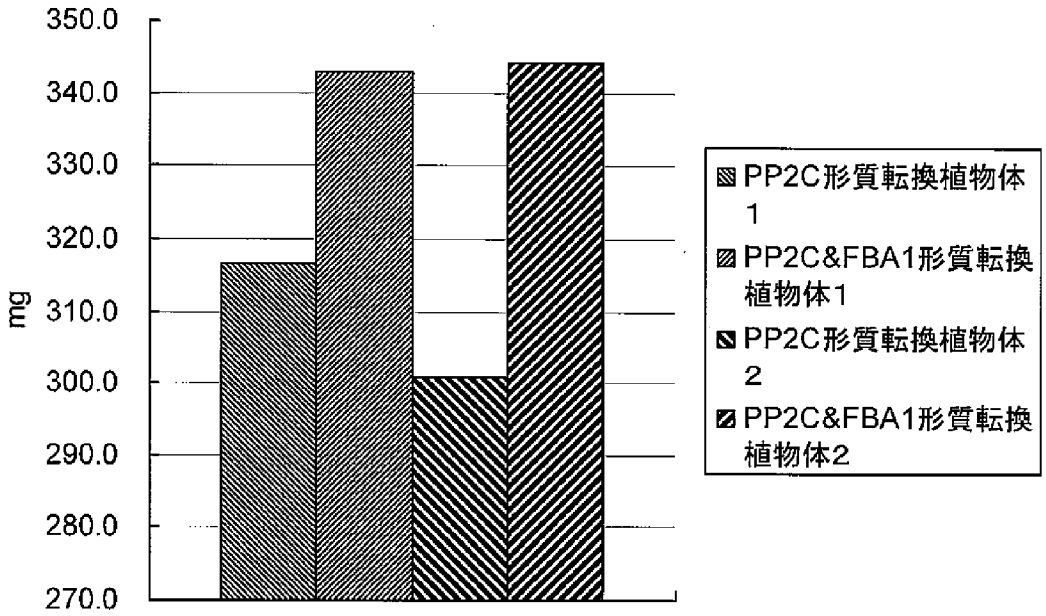
[図7]


160
140
120
100
80
60
40
20
0
mg
野生型
形質転換植物体1
形質転換植物体2
種子量

[図8]

PP2C 形質転換植物体 （参考例1）	野生型 (シロイヌナズナ)
PP2C&FBA1 形質転換植物体 （実施例1）	PP2C&FBA1 形質転換植物体 （実施例1）

[図9]


350.0
340.0
330.0
320.0
310.0
300.0
290.0
280.0
270.0
mg
PP2C形質転換植物体1
PP2C&FBA1形質転換植物体1
PP2C形質転換植物体2
PP2C&FBA1形質転換植物体2