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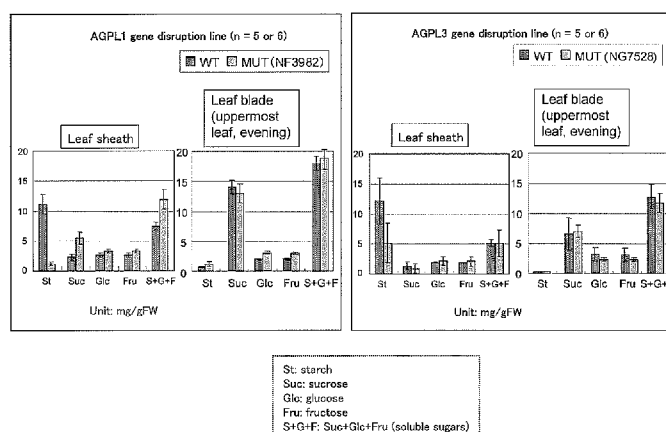
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(54) Title: PLANT VARIANT, METHOD FOR PRODUCING PLANT VARIANT, AND METHOD FOR ACCUMULATING SOLUBLE SUGAR

[Fig. 1]



(57) Abstract: This invention is intended to allow accumulation of large quantities of soluble sugars in tissue other than plant seeds. A plant is modified so as to suppress a gene encoding a subunit exhibiting the highest sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting.

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Description

Title of Invention: PLANT VARIANT, METHOD FOR PRODUCING PLANT VARIANT, AND METHOD FOR ACCUMULATING SOLUBLE SUGAR

Technical Field

[0001] The present invention relates to a plant variant lacking a given gene function, a method for producing such plant variant, and a method for accumulating a soluble sugar that regulates the amount of soluble sugar contained in a plant lacking a given gene function.

Background Art

[0002] ADP-glucose pyrophosphorylase (hereafter, referred to as "AGPase") is a heterotetramer comprising large subunits and small subunits encoded by different genes, and it is an enzyme associated with the starch synthesis pathway. Corn with lowered AGPase activity has heretofore been used for sweet corn breeding and its high sucrose content in seeds (albumens) has been known (Non-Patent Document 1). Also, Patent Document 1 discloses a method for increasing the seed yield of corn by inducing AGPase to express in a stage- or site-specific manner.

[0003] Non-Patent Document 2 discloses that inhibition of AGPaseB gene expression via RNAi in potato results in decreased starch content in the tuberous root and in increased sucrose content to a level approximately 10 times greater than that of a wild-type plant. Further, Patent Document 2 discloses that inhibition of AGPase activity in garden pea results in increased sucrose content in beans. Regarding garden pea, Non-Patent Document 3 also discloses that decreased starch content and increased sucrose content were observed in mutant garden pea germs exhibiting approximately 3% to 5% AGPase activity.

[0004] Further, Non-Patent Documents 4 and 5 each disclose the correlation between decreased starch content and lack of AGPase activity in *Arabidopsis thaliana*.

[0005] Furthermore, Non-Patent Document 6 discloses the floury 2 mutant that affects expression of a plurality of enzymes associated with starch biosynthesis, such as DBE or AGPase, in rice. Regarding rice-derived AGPase, four types of genes (i.e., AGPL1, AGPL2, AGPL3, and AGPL4 genes) are known as genes encoding large subunits, and two types of genes (i.e., AGPS1 and AGPS2 genes) and two types of AGPS2 gene transcription products (i.e., AGPS2a and AGPS2b) are known as genes encoding small subunits. Non-Patent Document 7 discloses that the seed amount is increased in rice into which *E. coli*-derived mutant AGPase (Pi-insensitive or constitutively active) has been introduced.

- [0006] As disclosed in Patent Documents 3 and 4, regarding modification of AGPase activity, it is known that introduction of site-directed mutation into the AGPS gene encoding the small subunit results in attenuated sensitivity to inorganic phosphate (Pi) and increased seed weight or amount. Thus, the AGPase activity of a plant was known to influence traits such as seed weight or amount.

Citation List

Patent Literature

- [0007] PTL 1: US 7,193,130
PTL 2: US 5,498,831
PTL 3: EP 1461431
PTL 4: US 2003/0177533

Non Patent Literature

- [0008] NPL 1: K. Lertrat, Int. J. Plant Breed., 2007, 1 (1), 27-30
NPL 2: B. Muller-Rober, EMBO J., 1992, 11: 1229-1238
NPL 3: A. M. Smith., Plant Phys., 1989, 89 (4), 1279-1284
NPL 4: T. P. Lin, Plant Phys., 1988, 86, 1175-1181
NPL 5: T. P. Lin, Plant Phys., 1988, 86, 1131-1135
NPL 6: H. Satoh, Journal of Applied Glycoscience, 2003, 50, 225-230
NPL 7: Yasuko S., Nagai, Plant Cell Phys., 2009, 50, 635-643

Summary of Invention

Technical Problem

- [0009] No techniques that regulate content of a soluble sugar such as sucrose in plant tissue other than seeds were known. Accordingly, it is an object of the present invention to provide a technique that enables production of plants capable of accumulating large quantities of soluble sugars in plant stems and having high soluble sugar content.

Solution to Problem

- [0010] The present inventors have conducted concentrated studies in order to attain the above object. As a result, they discovered that suppression of a given AGPase gene in a plant enables accumulation of large quantities of soluble sugars in the stem. This has led to the completion of the present invention.

The present invention includes the following.

- (1) A plant variant, in which the gene encoding a subunit having high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant is modified to be suppressed.
- (2) The plant variant according to (1), which is derived from a plant other than rice.
- (3) The plant variant according to (1), wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.

- (4) The plant variant according to (1), wherein the plant is a monocotyledonous plant.
- (5) The plant variant according to (1), wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- (6) A method for producing a plant variant comprising a step of modification to suppress the gene encoding a subunit having high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant.
- (7) The method for producing a plant variant according to (6), wherein the plant is derived from a plant other than rice.
- (8) The method for producing a plant variant according to (6), wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.
- (9) The method for producing a plant variant according to (6), wherein the plant is a monocotyledonous plant.
- (10) The method for producing a plant variant according to (6), wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- (11) The method for producing a plant variant according to (6), which further comprises steps of cultivating the progeny plant after modification and selecting a line with a fixed trait that enables accumulation of large quantities of soluble sugars.
- (12) A method for accumulating soluble sugars comprising preparing a plant variant modified so as to suppress the gene encoding a subunit having high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant and growing the plant variant under conditions that allow photosynthesis.
- (13) The method for accumulating soluble sugars according to (12), wherein the plant is derived from a plant other than rice.
- (14) The method for accumulating soluble sugars according to (12), wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.
- (15) The method for accumulating soluble sugars according to (12), wherein the plant is a monocotyledonous plant.
- (16) The method for accumulating soluble sugars according to (12), wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- (17) The method for accumulating soluble sugars according to (12), which further comprises steps of cultivating the progeny plant after modification and selecting a line

with a fixed trait that enables accumulation of large quantities of soluble sugars.

Advantageous Effects of Invention

- [0011] According to the present invention, large quantities of soluble sugars can be accumulated in a plant stem by suppressing a given gene, and a plant with high soluble sugar content can be produced. A plant produced in accordance with the present invention contains large quantities of soluble sugars and it thus can be used as a starting material for biofuels.

Brief Description of Drawings

- [0012] [fig.1] Fig. 1 is a characteristic diagram showing the results of quantification of the amounts of soluble sugars and starch accumulated in the leaf sheaths and in the leaf blades of a variant resulting from AGPL1 gene disruption and of a variant resulting from AGPL3 gene disruption.

Description of Embodiments

- [0013] Hereafter, the method for producing a plant containing large quantities of soluble sugars according to the present invention is described in detail.
- [0014] According to the present invention, a gene encoding a subunit exhibiting the highest sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase (AGPase) of a plant is suppressed, in order to accumulate large quantities of soluble sugars in a plant. The term "soluble sugars" used herein collectively refers to sugars dissolved in water, such as sucrose, glucose, and fructose. Accordingly, the expression "large quantities of soluble sugars are accumulated" refers to a case in which large quantities of any one of or a plurality of sugar components (i.e., sucrose, glucose, and fructose) are accumulated. The expression "large quantities of...accumulated" refers to a situation in which the amount of soluble sugars accumulated in a plant before the target gene is suppressed is increased to a significant level after the target gene has been suppressed.
- [0015] In the present invention, "suppression of a gene" refers to disruption of the gene of interest, suppression or lowering of the expression level of such gene, and inhibition of functions of a protein encoded by such gene. The term "disruption of the gene" refers to deletion of a region comprising part of or the entire coding region of the gene from the chromosome and disruption of the gene via introduction of a transposon or the like into the coding region of the gene. A method for lowering the gene expression level is not particularly limited. Examples thereof include a method in which the expression-regulated region of the gene is modified so as to reduce the extent of transcription and a method in which a transcription product of the gene is selectively degraded.
- [0016] Examples of gene suppression techniques that can be employed in the present invention include the so-called transposon method, the transgene method, the post-

transcriptional gene silencing method, the RNAi technique, the nonsense-mediated decay (NMD) method, the ribozyme method, the anti-sense method, the miRNA (micro-RNA) method, and the siRNA (small interfering RNA) method.

[0017] The gene to be suppressed in the present invention is a gene encoding a subunit exhibiting the highest sequence similarity with the subunit encoded by the AGPL1 gene of rice (it may be referred to as "Os05g0580000") among subunits constituting ADP-glucose pyrophosphorylase. When a plurality of isomers are identified as subunits constituting AGPase in a given plant, specifically, the plant genome is modified so as to suppress the gene encoding a subunit exhibiting the highest sequence similarity with the subunit encoded by the AGPL1 gene of rice. SEQ ID NOs: 1 and 2 show the nucleotide sequence of the AGPL1 gene of rice and the amino acid sequence of the subunit encoded by the AGPL1 gene, respectively. "Sequence similarity" is a value that quantifies the similarity between two amino acid sequences, and it is determined with the use of sequence similarity search software, such as BLAST, PSI-BLAST, or HMMER, under default settings. The gene to be suppressed in the present invention can be a gene having an amino acid sequence having 70% or higher, preferably 80% or higher, more preferably 90% or higher, and most preferably 95% or higher similarity with the amino acid sequence as shown in SEQ ID NO: 2 and encoding a subunit of AGPase. Here, the value of similarity is determined with the use of a database storing a computer program and gene sequence information provided with the BLAST (Basic Local Alignment Search Tool) program under default settings.

[0018] In other words, the term "the gene to be suppressed in the present invention" is synonymous with the homologous gene of the AGPL1 gene of rice, provided that the plant of such gene is not rice. Homologous genes originating from organisms other than rice are not particularly limited, and such genes can be identified by searching a database storing sequence information of various organism species. Specifically, the nucleotide sequence and the amino acid sequence as shown in SEQ ID NOs: 1 and 2 may be employed as query sequences to search, for example, the international nucleotide sequence database (e.g., DDBJ/EMBL/GenBank) or the SWISS-PROT database. Thus, the gene of interest can be easily searched for and identified from known databases.

[0019] The term "homologous genes" generally refers to genes that have evolved and diverged from a common ancestral gene, and the term also refers to homologous genes developed between two species (i.e., orthologs) and homologous genes developed within a single species via duplicate branching (i.e., paralogs). In other words, homologous genes, such as orthologs and paralogs, are within the scope of the "homologous genes" described above.

[0020] When a gene of a given plant is modified so as to accumulate large quantities of

soluble sugars in which the genomic information of the plant of interest is not known, a genome library or cDNA library may be prepared in accordance with a conventional technique, and hybridization may be carried out using the entire or part of the AGPL1 gene of rice as a probe. Thus, the gene to be suppressed can be identified.

- [0021] When a plant that is modified so as to accumulate large quantities of soluble sugars is rice, the AGPL1 gene is to be suppressed. Four types of genes; i.e., AGPL1, AGPL2, AGPL3, and AGPL4 genes, are known as subunits constituting rice AGPase. Traits are changed to accumulate large quantities of soluble sugars via suppression of the AGPL1 gene among such genes; however, such trait change cannot be observed when a gene of a different type is suppressed. As described in the examples below, a plant variant resulting from AGPL3 gene suppression accumulated soluble sugars in an amount substantially the same as that in a wild-type plant. SEQ ID NOs: 3 and 4 show the nucleotide sequence of the AGPL3 gene and the amino acid sequence of a subunit encoded by the AGPL3 gene, respectively.
- [0022] Based on the findings described above, it is preferable to identify a gene exhibiting higher sequence similarity with the nucleotide sequence and the amino acid sequence of the AGPL1 gene as shown in SEQ ID NOs: 1 and 2 than with the nucleotide sequence and the amino acid sequence of the AGPL3 gene as shown in SEQ ID NOs: 3 and 4, when a plant other than rice is modified so as to accumulate large quantities of soluble sugars. As is apparent from the examples below, suppression of a gene exhibiting comparatively higher sequence similarity with the nucleotide sequence and the amino acid sequence of the AGPL1 gene enables production of a plant variant capable of accumulating large quantities of soluble sugars.
- [0023] Any plants may be modified to accumulate large quantities of soluble sugars without particular limitation. Examples of target plants include, but are not limited to, dicotyledonous plants and monocotyledonous plants, such as plants belonging to Brassicaceae, Gramineae, Solanaceae, Leguminosae, and Salicaceae (see the examples below).
- [0024] Brassicaceae: *Arabidopsis thaliana*, *Brassica rapa*, *Brassica napus*, *Brassica oleracea* var. *capitata*, *Brassica rapa*, *Brassica napus*, *Brassica rapa* var. *chinensis*, *Brassica rapa* var. *rapa*, *Brassica rapa* var. *hakabura*, *Brassica rapa* var. *lancinifolia*, *Brassica rapa* var. *peruviridis*, *Brassica rapa* var. *chinensis*, *Brassica raphanus sativus*, *Wasabia japonica*, etc.
- [0025] Solanaceae: *Nicotiana tabacum*, *Solanum melongena*, *Solaneum tuberosum*, *Lycopersicon lycopersicum*, *Capsicum annuum*, *Petunia*, etc.
- [0026] Leguminosae: *Glycine max*, *Pisum sativum*, *Vicia faba*, *Wisteria floribunda*, *Arachis hypogaea*, *Lotus corniculatus* var. *japonicus*, *Phaseolus vulgaris*, *Vigna angularis*, *Acacia*, etc.

- Compositae: *Chrysanthemum morifolium*, *Helianthus annuus*, etc.
- [0027] Palmae: *Elaeis guineensis*, *Elaeis oleifera*, *Cocos nucifera*, *Phoenix dactylifera*, *Copernicia*, etc.
- [0028] Anacardiaceae: *Rhus succedanea*, *Anacardium occidentale*, *Toxicodendron vernicifluum*, *Mangifera indica*, *Pistacia vera*, etc.
- [0029] Cucurbitaceae: *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Cucumis sativus*, *Trichosanthes cucumeroides*, *Lagenaria siceraria* var. *gourda*, etc.
- [0030] Rosaceae: *Amygdalus communis*, *Rosa*, *Fragaria*, *Prunus*, *Malus pumila* var. *domestica*, etc.
- Caryophyllaceae: *Dianthus caryophyllus*, etc.
- Salicaceae: *Populus trichocarpa*, *Populus nigra*, *Populus tremula*, etc.
- Myrtaceae: *Eucalyptus camaldulensis*, *Eucalyptus grandis*, etc.
- [0031] Gramineae: *Zea mays*, *Oryza sativa*, *Hordeum vulgare*, *Triticum aestivum*, *Phyllostachys*, *Saccharum officinarum*, *Pennisetum purpureum*, *Erianthus ravenae*, *Miscanthus virgatum*, *Sorghum*, *Panicum*, etc.
- Liliaceae: *Tulipa*, *Lilium*, etc.
- [0032] Production of monocotyledonous plants capable of accumulating large quantities of soluble sugars is particularly preferable. Among monocotyledonous plants, plants of Gramineae, such as rice, wheat, barley, sugar cane, and corn, are particularly preferable.
- [0033] In particular, plants resulting from suppression of a given gene according to the present invention exhibit traits that enable accumulation of larger quantities of soluble sugars compared with original plants without suppression of the gene. In general, excess sugar components (soluble sugars) photosynthesized in leaves are translocated to an organ that accumulates excess substances resulting from photosynthesis, such as a stem, and they are accumulated therein. Thus, plants resulting from suppression of a given gene according to the present invention exhibit traits that enable accumulation of large quantities of soluble sugars in an organ that accumulates excess substances resulting from photosynthesis, such as a stem. In the case of rice, the leaf sheath and the culm (stem) function as organs that accumulate excess substances resulting from photosynthesis. Specifically, large quantities of soluble sugars are accumulated in the leaf sheath and the culm (stem) in the case of rice.
- [0034] According to the production method of the present invention, accordingly, plants capable of accumulating large quantities of soluble sugars can be produced. Since such plants have accumulated large quantities of soluble sugars, such plants can be effectively used as starting materials for biofuels. Specifically, the plants produced according to the present invention have accumulated large quantities of soluble sugars and thus can be used for production of biofuels utilizing microorganisms.

- [0035] In the past, it was difficult to evaluate changes in the accumulation of sucrose in the organs of model plants such as *Arabidopsis thaliana* and rice as described above, because such plants accumulate fats or starch. Since the plants produced according to the present invention accumulate large quantities of soluble sugars in organs such as stems or leaf sheaths, the amount of soluble sugars contained in such organs may be used as an indicator to screen for plants producing increased amounts of soluble sugars, such as sucrose, or causal genes used for production of increased amounts of soluble sugars.
- [0036] In addition, livestock animals have heretofore been raised with the use of fermented roughage utilizing rice straw or the like. In general, fermented roughage is prepared by adding a sugar component to a plant (e.g., rice straw) to perform lactic acid fermentation with the aid of lactic bacteria. When the plants produced in accordance with the present invention are used as starting materials for fermented roughage, addition of a sugar component for lactic acid fermentation becomes unnecessary because such plants have accumulated large quantities of soluble sugars, as described above. With the utilization of the plants produced in accordance with the present invention, inexpensive and highly nutritional fermented roughage for livestock animals can be produced.
- [0037] When soluble sugars accumulated in organs that accumulate excess substances resulting from photosynthesis, such as leaf sheaths and stems, are used, it is preferable that plants be used before ear emergence. This is because soluble sugars accumulated in organs that accumulate excess substances resulting from photosynthesis are translocated to the ears in the form of sucrose in plants after ear emergence.

Other steps

As described above, plants may be modified so as to suppress a given gene, and progeny plants can then be obtained in accordance with a conventional technique. That is, progeny plants sustaining fixed traits that allow accumulation of large quantities of soluble sugars acquired via modification of plants so as to suppress a given gene can be obtained in accordance with a conventional technique.

- [0038] In the present invention, the term "plant body" refers to a grown individual plant, plant cell, plant tissue, callus, or seed. That is, a substance that can be grown to result in an individual plant in the end is regarded as a plant body in the present invention. Plant cells may be in various forms. Examples of plant cells include suspension cultured cells, protoplasts, and leaf slices. Plant bodies can be obtained by multiplying and differentiating such plant cells. Plant bodies can be reproduced from plant cells via a conventional technique in accordance with plant cell type.

Examples

[0039] Hereafter, the present invention is described in greater detail with reference to the examples, although the technical scope of the present invention is not limited to the examples below.

[0040] In this example, the capacity of variants resulting from disruption of subunits constituting rice AGPase to accumulate soluble sugars was examined using a rice model. In this example, variants resulting from disruption of subunits encoded by the *AGPL1* gene and variants resulting from disruption of subunits encoded by the *AGPL3* gene among subunits constituting rice AGPase were obtained. These variants were provided by the National Institute of Agrobiological Sciences as the collection of Tos17 mutant lines of a rice variety (Nipponbare).

In this example, the two rice seed lines shown below were provided.

[Table 1]

Tos17 line	Gene	Enzymatic function
NF3982	<i>AGPL1</i>	ADP-Glc Pyrophosphorylase
NG7528	<i>AGPL3</i>	ADP-Glc Pyrophosphorylase

[0041] Specifically, M1 seeds of the mutant lines (NF3982, NC7528, and NC0371) prepared via translocation of a rice endogenous retrotransposon Tos17 provided by the National Institute of Agrobiological Sciences were cultivated in pots in a greenhouse.

[0042] DNA was extracted from leaf blades of seedlings, and genotypes were inspected via PCR to determine whether or not NF3982 lacks *AGPL1*, NF7528 lacks *AGPL3*, and NC0371 lacks *ISA3*.

[0043] DNA was extracted in the following manner. At the outset, leaf blades grounded with the use of a Multi-Beads Shocker in an extraction buffer (1 M KCl, 100 mM Tris-HCl (pH 8), 10 mM EDTA) were allowed to stand at 70 degrees C for 20 minutes or longer. The grounded leaf blades were centrifuged at 15,000 rpm and room temperature for 10 minutes, and 150 microliters of the supernatant was added to the same amount of isopropanol. The resultant was centrifuged at 3,000 rpm and 4 degrees C for 30 minutes, and a precipitate was obtained. To the precipitate, 200 microliters of 70% ethanol was added, and the resultant was centrifuged at 3,000 rpm and 4 degrees C for 5 minutes. Thereafter, the supernatant was discarded and the precipitate was air-dried at room temperature for 1 to 2 hours. The air-dried precipitate was dissolved in 1/10 TE and used for PCR.

[0044] PCR was carried out using the TaKaRa Multiplex PCR Assay Kit. The reaction was carried out by repeating a cycle of 94 degrees C for 60 seconds, 94 degrees C for 30 seconds, 58 degrees C for 90 seconds, and 72 degrees C for 90 seconds 35 times, followed by 72 degrees C for 10 minutes. Primers shown in Table 2 were used, and forward and reverse primers of such lines and the Tos17-tail3 primer were used at the

same time.

[Table 2]

Line or primer name	Forward or Reverse	Sequence	SEQ ID NO:
NF3982	Forward	CCTCTCTCCAACAGGAGCAC	SEQ ID NO: 5
	Reverse	ATGATTTGCGTGGTTGTTGA	SEQ ID NO: 6
NG7528	Forward	TCCATGTAGTCCATGCGGTA	SEQ ID NO: 7
	Reverse	GCGGTGAGTACAGCGTACAA	SEQ ID NO: 8
NC0371	Forward	GTTGGTATTTGTCCGAGCGT	SEQ ID NO: 9
	Reverse	TTCGTCACAGCAACCCAATA	SEQ ID NO: 10
Tos17-tail3	–	GAGAGCATCATCGGTTACATCTTCTC	SEQ ID NO: 11

M2 seeds of WT lines and homozygous KO lines obtained from plants the genotypes of which had been confirmed were seeded, 6 individuals from each line were cultivated in 1/5000-a pots in a greenhouse, and the resultants were subjected to the measurement of sugar and starch concentration and enzyme activity. In the greenhouse, a light period of 14 hours from 5:00 to 19:00 was set, and temperature was set at 27 degrees C during the light period and 23 degrees C during the dark period.

[0045] Sugar and starch concentration was measured using an F-kit (J. K. International), and measurement was carried out with the use of microplate spectrophotometers (Viento XS, Dainippon Sumitomo Pharma Co., Ltd.). At the outset, 1,000 microliters of 80% ethanol was added to about 50 mg of the test material grounded in liquid nitrogen, and the mixture was thoroughly agitated. Thereafter, the mixture was incubated at 80 degrees C for 10 minutes, the resultant was centrifuged at 10,000 rpm and room temperature for 5 minutes, and the supernatant was recovered. To the remaining precipitate, 500 microliters of 80% ethanol was added, and the same procedure was carried out. The thus-obtained supernatant was dehydrated with a centrifugal evaporator, 500 microliters of distilled water was added thereto, and the mixture was thoroughly agitated. The resultant was centrifuged at 15,000 rpm and 4 degrees C for 5 minutes, and the supernatant was recovered as an extract. Concentration of each sugar (mg/ml) in the extract was measured in accordance with the procedure of the F-kit, and sugar concentration per fresh weight was determined based on formula (1).

[0046] Formula (1): sugar concentration (mg/gFW) = sugar concentration (mg/ml) x 0.5 (ml)/ fresh weight (g)

The results of quantitative analysis of sugar and starch concentration are shown in Fig. 1. In Fig. 1, the term "AGPL1 gene disruption line" refers to a variant of the NF3982 line, and the term "AGPL3 gene disruption line" refers to a variant of the NG7528 line. In Fig. 1, "St" indicates starch, "Suc" indicates sucrose, "Glc" indicates

glucose, "Fru" indicates fructose, and "S+G+F" indicates a total of sucrose, glucose, and fructose (i.e., soluble sugars).

[0047] The results shown in Fig. 1 demonstrate that the amount of sucrose accumulated in the leaf sheath of a variant resulting from AGPL1 gene disruption increased to a level 2.4 times greater than that in a wild-type line, and the amount of soluble sugars accumulated in the leaf sheath thereof was significantly increased, compared with that in a wild-type line. In addition, the amount of starch accumulated in the leaf sheath of a variant resulting from AGPL1 gene disruption was significantly decreased, compared with that in a wild-type line. In contrast, the amount of soluble sugars such as sucrose, and starch accumulated in the leaf blade of a variant resulting from AGPL1 gene disruption was substantially the same as that in wild-type plants. In addition, the amount of soluble sugars, such as sucrose, and starch accumulated in the leaf sheath and the leaf blade of a variant resulting from AGPL3 gene disruption was substantially the same as that in wild-type plants.

[0048] The results demonstrate that disruption of a subunit encoded by the AGPL1 gene among subunits constituting rice AGPase enables accumulation of large quantities of soluble sugars in the leaf sheath.

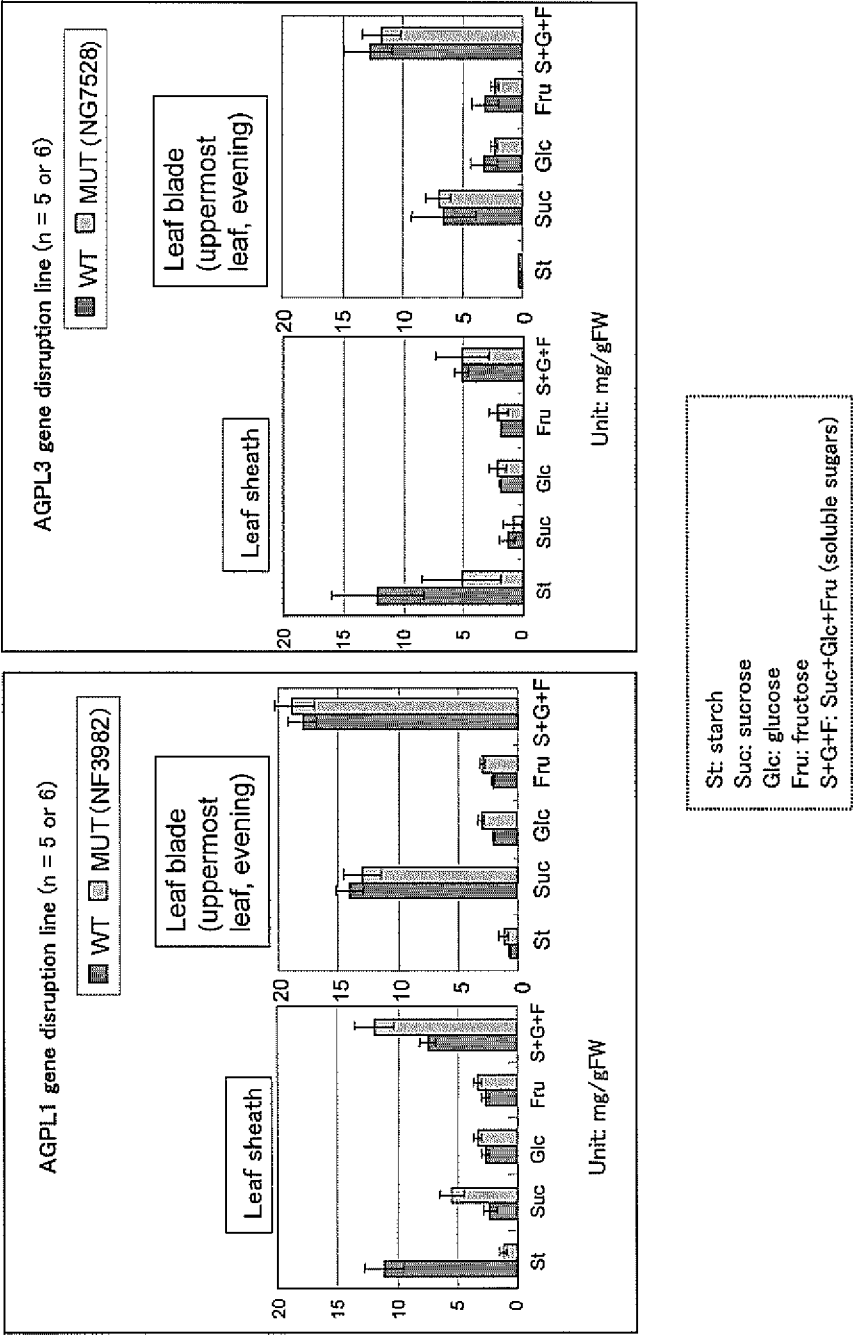
Claims

- [Claim 1] A plant variant, in which the gene encoding a subunit having high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant is modified to be suppressed.
- [Claim 2] The plant variant according to claim 1, which is derived from a plant other than rice.
- [Claim 3] The plant variant according to claim 1, wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.
- [Claim 4] The plant variant according to claim 1, wherein the plant is a monocotyledonous plant.
- [Claim 5] The plant variant according to claim 1, wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- [Claim 6] A method for producing a plant variant comprising a step of modification to suppress the gene encoding a subunit having high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant.
- [Claim 7] The method for producing a plant variant according to claim 6, wherein the plant is derived from a plant other than rice.
- [Claim 8] The method for producing a plant variant according to claim 6, wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.
- [Claim 9] The method for producing a plant variant according to claim 6, wherein the plant is a monocotyledonous plant.
- [Claim 10] The method for producing a plant variant according to claim 6, wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- [Claim 11] The method for producing a plant variant according to claim 6, which further comprises steps of cultivating the progeny plant after modification and selecting a line with a fixed trait that enables accumulation of large quantities of soluble sugars.
- [Claim 12] A method for accumulating soluble sugars comprising preparing a plant variant modified so as to suppress the gene encoding a subunit having

high sequence similarity with the subunit encoded by the AGPL1 gene of rice among subunits constituting ADP-glucose pyrophosphorylase of a plant and growing the plant variant under conditions that allow photosynthesis.

- [Claim 13] The method for accumulating soluble sugars according to claim 12, wherein the plant is derived from a plant other than rice.
- [Claim 14] The method for accumulating soluble sugars according to claim 12, wherein the AGPL1 gene encodes the AGPase subunit comprising the amino acid sequence as shown in SEQ ID NO: 2.
- [Claim 15] The method for accumulating soluble sugars according to claim 12, wherein the plant is a monocotyledonous plant.
- [Claim 16] The method for accumulating soluble sugars according to claim 12, wherein the soluble sugar content in an organ that accumulates excess substances resulting from photosynthesis is significantly higher than the content thereof before modification.
- [Claim 17] The method for accumulating soluble sugars according to claim 12, which further comprises steps of cultivating the progeny plant after modification and selecting a line with a fixed trait that enables accumulation of large quantities of soluble sugars.

[Fig. 1]



INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2012/001985

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/10 C12N15/82 C12N9/12
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C12Y

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROSTI SANDRINE ET AL: "A mutant of rice lacking the leaf large subunit of ADP-glucose pyrophosphorylase has drastically reduced leaf starch content but grows normally", FUNCTIONAL PLANT BIOLOGY, vol. 34, no. 6, 2007, pages 480-489, XP009160827, ISSN: 1445-4408 abstract page 481, left-hand column, line 1 - line 17 page 485, right-hand column, line 20 - line 22 ----- -/--	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 July 2012

Date of mailing of the international search report

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Mundel, Christophe

INTERNATIONAL SEARCH REPORT

International application No

PCT/JP2012/001985

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUNG-IL PARK ET AL: "Modification of Sugar Composition in Strawberry Fruit by Antisense Suppression of an ADP-glucose Pyrophosphorylase", MOLECULAR BREEDING, KLUWER ACADEMIC PUBLISHERS, DO, vol. 17, no. 3, 1 April 2006 (2006-04-01), pages 269-279, XP019258789, ISSN: 1572-9788	1,2,5-7, 10-13, 16,17
Y	abstract page 270, right-hand column, line 34 - line 37 figure 3 page 278, left-hand column, line 34 - line 43 page 278, right-hand column, line 33 - line 43	3,4,8,9, 14,15
X	----- WEBER HANS ET AL: "Antisense-inhibition of ADP-glucose pyrophosphorylase in developing seeds of Vicia narbonensis moderately decreases starch but increases protein content and affects seed maturation", PLANT JOURNAL, vol. 24, no. 1, October 2000 (2000-10), pages 33-43, XP002679365, ISSN: 0960-7412	1,2,5-7, 10-13, 16,17
Y	abstract page 34, right-hand column, line 9 - line 19 AGP-antisense expression increases sucrose and seed water and extends the filling period.; page 40	3,4,8,9, 14,15
X	----- MUELLER-ROEBER B ET AL: "INHIBITION OF THE ADP-GLUCOSE PYROPHOSPHORYLASE IN TRANSGENIC POTATOES LEADS TO SUGAR-STORING TUBERS AND INFLUENCES TUBER FORMATION AND EXPRESSION OF TUBER STORAGE PROTEIN GENES", EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 11, no. 4, 1 January 1992 (1992-01-01), pages 1229-1238, XP002058763, ISSN: 0261-4189	1,2,5-7, 10-13, 16,17
Y	abstract page 1230, left-hand column, line 5 - line 11 page 1231, right-hand column, line 20 - line 22 page 1234, right-hand column, line 20 - line 25	3,4,8,9, 14,15
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INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2012/001985

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SANG-KYU LEE ET AL: "Identification of the ADP-glucose pyrophosphorylase isoforms essential for starch synthesis in the leaf and seed endosperm of rice (<i>Oryza sativa</i> L.)", PLANT MOLECULAR BIOLOGY, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 65, no. 4, 4 April 2007 (2007-04-04), pages 531-546, XP019556067, ISSN: 1573-5028, DOI: 10.1007/S11103-007-9153-Z the whole document -----	1-17
Y	AKIHIRO TAKASHI ET AL: "Gene expression of ADP-glucose pyrophosphorylase and starch contents in rice cultured cells are cooperatively regulated by sucrose and ABA", PLANT AND CELL PHYSIOLOGY, vol. 46, no. 6, June 2005 (2005-06), pages 937-946, XP002679366, ISSN: 0032-0781 the whole document -----	1-17