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(54) Titre : METHODE VISANT A ACCROITRE L'ACTIVITE DE LA GGT CHEZ LES PLANTES, PLANTES PRESENTANT UNE ACTIVITE ACCRUE DE LA GGT, ET METHODE VISANT A PRODUIRE DE TELLES PLANTES

(54) Title: A METHOD OF INCREASING THE GGT ACTIVITY OF PLANTS, AND PLANTS WITH INCREASED GGT ACTIVITY AND A METHOD OF PRODUCING SUCH PLANTS

(57) Abrégé/Abstract:
The object of the present invention is to provide a plant having increased activity of glutamate glyoxylate aminotransferase (GGT) and a method of producing the plant, to provide a seed of the plant, and to provide a plant having an increased amino acid content, particularly a plant improved in the content of at least one of the amino acid selected from the group consisting of serine, arginine, glutamine, and asparagine and a method of producing the plant, as well as the seed of the plant. A plant having increased GGT activity is produced by mutagenesis, introduction of a nucleic acid molecule and the like. A genetic construction being capable of enhancing the expression of a GGT gene, particularly a genetic construct being capable of expressing a GGT gene and/or increasing the level of an endogenous gene having GGT activity is introduced into a plant.

Abstract

The object of the present invention is to provide a plant having increased activity of glutamate glyoxylate aminotransferase (GGT) and a method of producing the plant, to provide a seed of the plant, and to provide a plant having an increased amino acid content, particularly a plant improved in the content of at least one of the amino acid selected from the group consisting of serine, arginine, glutamine, and asparagine and a method of producing the plant, as well as the seed of the plant. A plant having increased GGT activity is produced by mutagenesis, introduction of a nucleic acid molecule and the like. A genetic construction being capable of enhancing the expression of a GGT gene, particularly a genetic construct being capable of expressing a GGT gene and/or increasing the level of an endogenous gene having GGT activity is introduced into a plant.

A METHOD OF INCREASING THE GGT ACTIVITY OF PLANTS, AND PLANTS WITH INCREASED GGT ACTIVITY AND A METHOD OF PRODUCING SUCH PLANTS

BACKGROUND OF THE INVENTION

The present invention relates to plants having increased activity of glutamate glyoxylate aminotransferase (GGT).

The present invention also relates to methods of utilizing glutamate glyoxylate aminotransferase (GGT) and/or a gene encoding GGT.

The present invention also relates to methods of increasing the amino acid content of a plant and/or the seeds thereof, and more particularly, to methods of increasing the content of one or more amino acids selected from the group consisting of serine (Ser), arginine (Arg), glutamine (Gln) and asparagine (Asn), and relates to plants having increased content of amino acids, particularly, the plants having increased content of one or more amino acids selected from the group consisting of serine (Ser), arginine (Arg), glutamine (Gln), and asparagine (Asn), of the plants and/or the seeds thereof and to a method of producing such plants.

Furthermore, the present invention relates to the use of the plants and/or the seeds thereof obtained according to the present invention for producing foods or feeds, and the present invention also relates to foods or feeds containing such plants and/or their seeds.

In the photorespiration which metabolizes glycolate produced by the oxygenase activity of RuBisco, it has been thought that glycolate is metabolized to glyoxylate by glycolate oxygenase in peroxisomes, and this glyoxylate is further metabolized by at least two glyoxylate aminotransferases (Somerville: PNAS 77: 2684-2687, 1980). Although a peroxisomal glyoxylate aminotransferase gene has not been identified until now, Liepman et al. recently reported an alanine: glutamate glyoxylate aminotransferase localized in the peroxisomes functioning in the photorespiratory system of Arabidopsis thaliana (Plant J. 25: 487-498). However,

the glutamate glyoxylate aminotransferase gene was still unknown. In addition, it was not necessarily clarified what roles this glutamate glyoxylate aminotransferase activity plays in the plant characteristics including the content of various amino acids including glutamate, increase and decrease in total amino acid content, photosynthetic capacity, and stress tolerance. Moreover, a possibility to be able to improve the various characteristics of plants by manipulating proteins with a glutamate glyoxylate aminotransferase activity or the gene encoding for such proteins, particularly a possibility to be able to increase actually the content of total amino acids and/or the content of specified amino acids in plant or their seeds, has never been suggested in previous reports.

SUMMARY OF THE INVENTION

An object of the present invention is to provide plants having increased glutamate glyoxylate aminotransferase (GGT) activity and a method of preparing the plant, and to provide the seeds thereof.

An object of the present invention is also to provide plants having increased amino acid content, particularly those having increased content of one or more amino acids selected from the group consisting of serine (Ser), arginine (Arg), glutamine (Gln) and asparagine (Asn), as compared with the wild-type plants of the same species cultivated under the same condition, and a method of preparing such plants, and to provide the seeds of such plants.

Another object of the present invention is to provide new methods of utilizing GGT and the genes encoding GGT.

More specifically, an object of the present invention is to provide a method of utilizing the GGT and the gene coding for GGT for increasing the amino acid content of plants.

In addition, another object of the present invention is to provide feeds and/or foods containing plants and/or their seeds having increased contnt of amino acid, particularly those having increased content of one or more amino acids selected from the group consisting of Ser, Arg, Gln, and Asn, and the use of such

plants and/or their seeds for manufacturing of feeds or foods.

Moreover, an object of the present invention is to provide a method of producing plant extracts containing one or more amino acids selected particularly from the group consisting of Ser, Arg, Gln and Asn from the plants and/or their seeds having increased content of the above-mentioned amino acids, and to provide the use of the plants and/or their seeds having increased content of the above-mentioned amino acids for producing amino acids, particularly one or more amino acids selected from the group consisting of Ser, Arg, Gln and Asn.

In addition, another object of the present invention is to provide the utilization of plants and/or their seeds obtained according to the present invention as a ground for the production or material of other substances for which amino acids are used as starting materials.

The present invention relates to a plant in which glutamate glyoxylate aminotransferase (GGT) activity is increased as compared with the wild type plants of the same species.

Moreover, the present invention relates to a plant in which the transcription of a gene having GGT activity is increased as compared with the wild type plants of the same species.

In addition, the present invention also relates to a method of increasing the content of amino acids in plants, particularly the content of one or more amino acids selected from the group consisting of serine, arginine, glutamine and asparagine in the plant, which comprises increasing the GGT activity.

In addition, the present invention relates to a transgenic plant into which a gene construct capable of increasing the expression of GGT gene, particularly a gene construct capable of expressing the GGT gene and/or a gene construct capable of increasing the expression of genes with the endogenous GGT activity is introduced, wherein the GGT activity of the transgenic plants is increased as compared with the wild type plants of the same species or the corresponding non-transformed plants which was cultivated under the same condition.

Moreover, the present invention is also a method of increasing the GGT

activity of plants, which comprises introducing a gene construct capable of increasing the expression of the GGT gene, particularly, a gene construct capable of expressing the GGT gene and/or a gene construct capable of increasing the transcription of genes having the endogenous GGT activity.

The present invention is also a method of producing plants having an increased GGT activity, which comprises geminating the plants having increased GGT activity as compared with the wild type plants of the same species or the plant seeds having increased GGT activity as compared with the corresponding non-transformed plants, or regenerating plant bodies from the above mentioned plants or transformed plant cells, or by the proliferating the plants or transgenic plants by vegetative proliferation.

Particularly, according to the present invention, the GGT activity specifically means the GGT activity in peroxisomes.

As used herein, in comparison with a transgenic plant into which a genetic construct capable of increasing the expression of the GGT gene was introduced, the term "non-transgenic plants" means "plants into which a genetic construct capable of increasing the expression of the GGT gene was not introduced". These "plants into which a genetic construct capable of increasing the expression of the GGT gene was not introduced" include, in addition to wild type plants, the plants into which a genetic construct other than the genetic construct capable of increasing the expression of the GGT gene has been introduced. In addition, "a genetic construct capable of increasing the expression of the GGT gene" includes a gene construct capable of expressing the GGT gene, for example, a gene construct containing the GGT gene which is functionally linked to an appropriate promoter and a genetic construct capable of increasing the transcription of the GGT gene, for example, a construct containing an enhancer. The term a "genetic construct" as used herein means any construct capable of being inherited to the descendents in any form, particularly it means nucleic acid molecules. In the case where the genetic construct contains a gene, it may be specifically referred to as a "gene construct". Therefore, for example, "a

genetic construct" not only includes nucleic acid molecules containing a gene but also includes nucleic acid fragments containing a transcriptional activation element, an enhancer or the like.

More specifically, the present invention relates to plants in which the activity of GGT having the homology of 60% or more in the amino acid sequence to the amino acid sequence described in SEQ ID No. 2 or 4 is increased as compared with the wild type plant of the same species cultivated under the same condition.

In particular, the present invention relates to plants having increased GGT activity as compared with the wild-type plants cultivated under the same condition, wherein the GGT has the amino acid sequence described in SEQ ID No. 2 or 4.

Moreover, the present invention relates to transgenic plants into which a genetic construct containing a nucleotide sequence being capable of hybridizing with the polynucleotide described in SEQ ID No. 1 or 3 under a stringent condition is introduced, wherein the GGT activity of the transgenic plants is increased as compared with the corresponding non-transformed plants cultivated under the same condition.

In particular, the present invention relates to the transgenic plants into which a genetic construct containing the nucleotide sequence described in SEQ ID No. 1 or 3 is introduced, wherein the GGT activity of the transgenic plants is increased as compared with the corresponding non-transgenic plants cultivated under the same condition.

Moreover, the present invention is related to a method of increasing the content of amino acid, particularly, the content of one or more amino acids selected from the group consisting of Ser, Arg, Gln and Asn of plants and/or their seeds, the method comprising the step of preparing transgenic plants by introducing a gene construct capable of expressing GGT, wherein the gene construct is able to increase the GGT activity of the transgenic plants as compared with the corresponding non-transgenic plants cultivated under the same condition,

and to plants having increased content of total amino acids, particularly the plants and/or their seeds having increased content of one or more amino acids selected from the group consisting of Ser, Arg, Gln and Asn.

The GGT activity of the plants of the present invention is increased preferably about 1.2-fold or more, more preferably about 3-fold or more and most preferably about 5-fold or more, compared with the GGT activity level in the corresponding tissues of the wild-type plants, or non-transgenic plants, cultivated under the same condition.

According to one aspect of the present invention, there is provided a method of increasing an amino acid content in a plant or in a seed of the plant, said method comprising the step of introducing a genetic construct for the expression of a glutamate glyoxylate aminotransferase (GGT) gene into a cell of the plant to produce a transgenic plant, wherein:

- the GGT gene encodes a polypeptide having GGT activity in a peroxisome;
- the C-terminal of the polypeptide encoded by the GGT gene has an amino acid sequence of [Ser or Ala]-[Arg or Lys]-[Ile or Leu or Met];
- the complement sequence of the GGT gene has a nucleotide sequence which hybridizes, under a stringent condition, to the polynucleotide of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent condition comprises a washing step at 50°C in 2X SSC and 0.1% SDS;
- the genetic construct increases the GGT activity of the transgenic plant as compared with a corresponding non-transformed plant which is cultivated under the same condition;
- the content of at least one amino acids selected from the group consisting of serine, arginine, glutamine and asparagine in the transgenic plant is increased as compared with the corresponding non-transformed plant which is cultivated under the same condition.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the diagrammatic drawing of the photorespiration pathway in higher plants. The large arrow indicates the reaction catalyzed by glutamate glyoxylate aminotransferase.

Figure 2 shows the comparison of amino acid sequences of glutamate glyoxylate aminotransferase from *Arabidopsis thaliana*. The locations of the identical amino acids are indicated by asterisk.

Figure 3 shows the structure of the glutamate glyoxylate aminotransferase gene from *Arabidopsis thaliana* and the inserted location thereof in pBI101. Exons are shown as black boxes. The genomic 5089 bp region was amplified by PCR and cloned into pBI101 (-GUS/-NOS-ter) using BmaHI site on the genome and Hind III site on the primer. Using this vector, the clone was introduced into a GGT1 gene knockout line (ggt1-1) by way of Agrobacterium-mediated transformation.

Figure 4 shows the comparison between the growth of the control strain and the GGT1 introduced strain (ggt1-1/GGT1). The weight of the aboveground parts of 95 individual seedlings of each wild type non-transformed strain (Control) and the GGT1-introduced strain (ggt1-1/GGT1), both cultivated for 2 weeks under an ordinary culture condition, was measured, and the data were compared.

Figure 5 is a graph showing comparison at the GGT1 mRNA level between the control stain and the GGT1-introduced strain.

Figure 6 is a graph showing the comparison of the GGT enzyme activity level between the control stain and GGT1-introduced strain.

Figure 7 shows the results of measurement of the content of amino acids in the seedlings grown for 2 weeks on PNS medium under a light condition of 70μmol m^{-2} s^{-1}. (A): the content of major major acids (nmol/mg FW), and (B): the content of total amino acids of the seedling (nmol/mg FW).

Figure 8 shows the amino acid content of the rosette leaves of the plant body cultivated for 42 days on rock wools using PNS as a fertilizer under a light condition of 70μmol m^{-2} s^{-1}. (A): the content of main amino acids (nmol/mg FW), (B): the content of total amino acids (nmol/mg FW).

Figure 9 is a graph showing the comparison of the GGT1 mRNA level between the GGT1-introduced strains and the control strain.

Figure 10 is a graph showing the comparison of the GGT enzyme activity (A) and the HPR activity (B) of the GGT1-introduced strain and the control strain. Each enzyme activity of the control plant was considered as 1.

Figure 11 shows the results of measurement of the serine content of the seedlings cultivated for 2 weeks on PNS medium under a light condition of 70μmol m^{-2} s^{-1}.

Figure 12 shows the results of comparison of the GGT1 mRNA levels, the GGT enzyme activity levels and the Ser contents of the transgenic plant produced by introducing a construct for expressing GGT1 into the wild type strain and the control strain. The correlation coefficient and regression formula obtained are shown. (A): the relative GGT enzyme activity vs. the relative GGT1 mRNA level, (B): the Ser content vs. the relative GGT1 mRNA level, and (C): the Ser content vs. the relative GGT enzyme activity.

Figure 13 shows the results of measurement of the amino acid content of the seedlings grown for 2 weeks on 1/2 MS medium under a light condition of 70μmol m^{-2} s^{-1}. (A): the content of major amino acids, and (B): the content of total amino acids.

Figure 14 shows the amino acid content of the seeds obtained from the

plant bodies cultivated under continuous lighting (a condition of about 200 μmol m^{-2} s^{-1}) with the modified PNS fertilizer (5 mM KNO_3 was replaced by 2.5 mM NH_4NO_3)(n=4). The content of major amino acids (A), the content of arginine (B), and the content of total amino acids C), each in nmol/mg FW.

Figure 15 is the results of another experiment performed under the same condition as Figure 14 (n = 2). The content of major amino acids (A), the content of arginine (B), the content of total amino acids (C), each indicated in nmol/mg FW.

Figure 16 shows the amino acid homology between *Arabidopsis thaliana* GGT and the proteins which are suspected to be rice (*Oryza sativa)* GGT protein. GGT1: *Arabidopsis thaliana* GGT1, Japonica_GGT: suspected GGT protein from *Oryza sativa* japonica, and Indica_GGT: suspected GGT protein from *Oryza sativa* indica. The locations where all the amino acids are identical are indicated by asterisk.

Figure 17 is the results of measurement of the content of amino acids of the daytime leaves of primary transgenic rice plants into which the *Arabidopsis*-derived GGT gene was introduced. The numerical values are the relative values to the total amino acid content are shown. Major amino acids of which relative contents to the total were about 10% were selected and shown in the figure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The objects of the present invention may be achieved by selecting or preparing plants in which glutamate glyoxylate aminotransferase (GGT) activity is increased as compared with the wild type plants of the same species, or by selecting or preparing plants transgenic plants in which GGT activity is increased as compared with the corresponding non-transgenic plants.

For example, an object of the present invention may be achieved by increasing the expression of a glutamate glyoxylate aminotransferase (GGT) gene (GGT gene) by introducing a genetic construct capable of increasing the expression of a gene encoding GGT into plants. Such genetic constructs include

a genetic construct capable of expressing GGT, a genetic construct capable of expressing a transcription activating factor, a nucleic acid fragment with a function to increase the transcription activity, and the like.

In one embodiment of the present invention, a transgenic plant in which the expression of the gene coding for GGT is increased by introduction of a gene construct capable of expressing GGT, as compared with the corresponding non-transformed plants cultivated under the same condition, is selected.

In another embodiment of the present invention, the expression of the GGT gene is increased by increasing of the copy numbers of the GGT gene. In another embodiment of the invention, the transcription of GGT gene is increased by the expression, more preferably by the overexpression of a transcriptional activator, and the GGT activity is increased consequently. In one embodiment of the invention, the transcription of the GGT gene is increased by the introduction of an enhancer and the like including a cis-element having a transcription-activating function, and the GGT activity is increased consequently.

The term "glutamate glyoxylate aminotransferase" as used herein means the generic name of the proteins having the glutamate glyoxylate aminotransferase activity, namely, proteins possessing the activity catalyzing the reaction: glyoxylate + glutamate → glycine + α-ketoglutarate (Figure 1). In particular, such proteins include, for example, proteins having homology in the amino acid sequence of at least 60%, preferably about 70% or more and most preferably 90% or more, to the amino acid sequence described in SEQ ID No. 2 or 4. This homology can be calculated by using the programs well known to those skilled in the art, such as FASTA, together with standard parameters. For example, FASTA Versions 2.0, 3.0, 3.2 3.3, and the like are available together with the standard parameters from DNA · Data Bank of Japan (DDBJ/CIB) (http://www.ddbj.nig.ac.jp/Welcome-j.html), National Institute of Genetics.

Similarly, "the gene encoding GGT" or "the GGT gene" includes any genes encoding proteins having the glutamate glyoxylate aminotransferase activity. In particular, such genes include the genes having the nucleotide

sequence homology to the nucleotide sequence described in SEQ ID No. 1 or 3 of preferably 70% or more and more preferably about 90% or more. This homology can also be calculated by using, for example, the FASTA and the like which were mentioned above. The nucleic acid molecules having such a homology are also nucleic acid molecules that can be hybridized with the nucleic acid molecules having the sequence of SEQ ID No. 1 or 3 under a stringent condition. The proteins that are encoded by such gene include the proteins possessing the amino acid sequences having addition, substitution and deletion of amino acid sequences in the amino acid sequence described in SEQ ID No. 2 or 4.

The term "stringent condition" as used herein means the condition in which a specific hybrid is formed but non-specific hybrids are not formed. It is difficult to numerically express this condition definitely. However, the following conditions may be considered: for example: a condition in which a pair of highly homologous DNAs, for example, a pair of 70% or more homologous DNAs, hybridize, but a pair of DNAs with lower homology does not hybridize, or a hybridization condition where the washing condition of Southern hybridization is 50 ℃, 2 x SSC and 0.1% SDS, preferably 1 x SSC and 0.1% SDS, more preferably 0.1 x SSC and 0.1% SDS. Although the genes that can hybridize under such conditions may include the genes having stop codons or mutations at the active center, such genes can be easily eliminated by linking it to a commercially available activity-expressing vector and by measuring the GGT enzyme activity conventionally.

Thus, any genes or proteins, that have the gene sequence homology to SEQ ID No. 1 or 3, or having the amino acid sequence homology to SEQ ID No. 2 or 4 and that can be utilized as equivalently as these genes or proteins according to the present invention, for example, those derived from rice, are included. As such examples, the nucleotide sequence of the suspected GGT gene of *Oryza sativa* japonica, and the amino acid sequence of the protein, which may be encoded by this gene, are described in SEQ ID Nos. 34 and 35, respectively, and, similarly, the gene sequence of the suspected GGT gene of *Oryza sativa* indica,

and the amino acid sequence of the protein, which may be encoded by this gene, are described in SEQ ID Nos. 36 and 37, respectively. Homology of the amino acid sequence between Arabidopsis GGT1 and these rice proteins is shown in Figure 16. It is obvious that the homology at the amino acid sequence level between GGT1 and the proteins from japonica and indica corresponding to the GGT1 is very high.

In addition, the GGT genes which can be used in the present invention may be either the isogenic genes derived from the plants to be transformed or the heterologous genes obtained from other sources.

The term "transgenic plant having an increased GGT activity compared with the corresponding non-transformed plants cultivated under the same condition" as used herein means the transgenic plant of which total GGT activity due to both of the inherent GGT gene of the corresponding non-transgenic plant and the GGT gene existing on the gene construct used for transformation is increased as compared with the GGT activity of the corresponding non-transformed plant cultivated under the same condition, namely, the plant which belongs to the same species as said transgenic plant and was not transformed by a GGT gene-expressing construct. It was already mentioned that, in comparison with the transgenic plant into which a gene construct capable of expressing GGT was introduced, the term, "non-transgenic plant", means the "the plant into which a gene construct capable of expressing GGT was not introduced".

The GGT activity may be increased either at the transcription level, translation level or post-translational modification level. For example, the GGT activity can be increased by introducing a gene construct capable of expressing GGT, and by controlling the upstream elements involved in the control of the GGT activity and/or transcription amount, such as the GGT expression regulatory element, translation regulatory element and post-translational regulatory element. More specifically, for example, the GGT activity can be increased by introducing a gene construct capable of expressing GGT in particular, by increasing the copy numbers of endogenous GGT gene, by introducing a transcriptional activator, by

introducing an enhancer elevating the transcription activity of the endogenous GGT gene, or the like. These methods are well known to those skilled in the art. For example, it is known that when the DREB1A gene is expressed under the control of the promoter of rd29A gene (stress-induced promoter), the expression of the target gene of DREB1 greatly increases in response to a stress, as compared with the wild type plants (Nature Biotechnology, 17 287-, 1999). It is also reported that a target gene could be identified by inserting an enhancer randomly for activating transcription and by selecting individuals having a characteristic trait among them (Plant J., 34, 741-750, 2003; Plant Physiol., 129, 1544-1446, 2002).

According to the present invention, the GGT activity of the transgenic plant of the present invention is increased preferably about 1.2-fold or more, more preferably about 3-fold or more and most preferably about 5-fold or more, as compared with the GGT activity of the corresponding tissues of non-transgenic plant cultivated under the same condition.

In addition, even at the mRNA level, the GGT mRNA level of the transgenic plant of the present invention increases up to preferably about 2-fold or more, more preferably about 5-fold or more and most preferably about 30-fold or more, as compared with the GGT mRNA level of the corresponding tissues of the non-transformed plant cultivated under the same condition. A strong positive correlation is observed between the GGT activity and the mRNA level in the plant of the present invention and the plants obtained according to the present invention.

A plant having increased GGT activity only in a specific tissues, for example a plant wherein the GGT activity is increased only in stems including tubers, leaves or in flowers and a method of producing such a plant are also included in the scope of the present inventions. Therefore, even if the increase in the total amino acids content, or the increase in the amino acid content of at least one of the amino acids selected from Ser, Arg, Gln and Asn is found only in a part of the plant, the plants or the methods are also within the scope of the present

inventions.

According to the present invention, the increase in the GGT activity preferably occurs in a peroxisome, particularly in a peroxisome of a photosynthesis tissue. The photosynthesis tissue may be the tissue which photosynthesizes under the conventional culture conditions or cultivation conditions including a leaf, a stem, a silique and the like.

The GGT genes used as the target in the present invention can also be obtained from various plants. For example, DNA base sequence information of GGT genes can be obtained by retrieving it from a database using "alanine aminotransferase" as a keyword. According to the sequence information, the full-length cDNA can be obtained by using RT-PCR, 5'-RACE or 3'-RACE. It is also possible to obtain the cDNA by screening cDNA libraries by hybridization with a suitable probe according to the known sequence information. The probes used for the screening can be prepared according to the amino acid sequence or nucleotide sequence of GGT.

According to the present invention, the GGT gene of which expression should be increased is preferably localized in peroxisomes, particularly peroxisomes in the photosynthesis tissues as described above. The localization of GGT in the peroxisomes can be deduced from the presence of N-terminal sequences or C terminal sequences characteristic to the proteins localized in the peroxisomes. Such sequences include, for example Arg-(Leu/Gln/Ile)-X5-His-Leu and the similar sequences as the N-terminal sequences, and (Ser/Ala)-(Arg/Lys)-(Ile/Leu/Met) and the similar sequences as the C-terminal sequences. A protein having the GGT activity may be connected to such N-terminal or C-terminal sequence which is characteristic to a peroxisome-localized protein. Additionally, to confirm the localization the resulting GGT gene may be fused to a reporter gene such as GFP or GUS while maintaining the localization to peroxisomes and the fused gene may be expressed in a cell and tested. Alternatively, a GGT having a tag may be expressed and detected by a specific antibody to confirm the localization.

The gene constructs for increasing the expression of GGT gene according to the present inventions may be generated by using a method well known by those skilled in the art. The promoter for expressing the GGT gene may be any promoter which can function in a plant. For example, a gene construct where the GGT expression is driven by a cauliflower mosaic virus (CaMV) 35S promoter (EMBO J. 6: 3901-3907, 1987), a maize ubiquitin promoter (Plant Mol. Biol. 18: 675-689, 1992), an actin promoter, a tubulin promoter, and the like. The high expression promoters are particularly preferable. The terminators may also be those which can function in a plant cell. For example, the terminator from CaMV or the terminator from nopaline synthase gene can be used. The GGT expression unit which may exist in a plant genome may be also used. A molecular biological means including the procedures for designing nucleic acid constructs, isolating them and determining the sequences thereof may be found in the literatures such as Sambrook et al., Molecular cloning-Laboratory manual, Edition 2, Cold Spring Harbor Laboratory Press. For preparing the nucleic acid constructs usable in the present invention, gene amplification procedures including PCR method may be required in some cases. As for such procedures, for example, F. M. Ausubel et al. (eds), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994) can be referred to.

The method of introducing the nucleic acid construct in the above-described embodiment is not particularly limited. Any method for introducing genes into plant cells or into plant bodies, known by those skilled in the art, can be selected depending on the hosts. For example, the Agrobacterium mediated gene introduction method, the electroporation method or a particle gun can be employed. When Agrobacterium is used, the sequence to be introduced is preferably inserted between the left and right T-DNA border sequences. The suitable design and construction of the transformation vectors thus based on T-DNA are well known in the art. Further, the conditions required for the infection of a specified plant with agrobacteria harboring such a nucleic acid construct are also well known in the art. As for such techniques and conditions, Cell Technology,

additional volume, "*Model Shokubutsu no Jikken Protocol; Ine, Shiroinunazuna Hen* (Experiment Protocol for Model Plants; Edition of Rice Plants and *Arabidopsis thaliana*) published by Shujunsha (1996) can be referred to.

Although the plant species to be subjected to the gene manipulation are not particularly limited, the plants species are preferably those which can be easily cultivated and transformed and the regeneration systems of which have been established. In addition to the plants having the above-described characteristic properties, plant species for which large-scale cultivation techniques have been established and which have a high utility value as foods, are preferred in the present invention. Those plants include, in addition to *Arabidopsis thaliana* as the model plant, rice, maize, wheat, sugar beet, cassava, spinach, cabbages, lettuce, salad, celery, cucumber, tomato, broad bean, soybean, adzuki bean, kidney bean and pea. These plants may be the naturally occurring plants or those that have already been received a genetic modification such as the plants where the expression of the intrinsic natural GGT gene is increased. The plants that have received any genetic modification may be selected from an existing library, for example from an existing high-expression library.

Then the genetically manipulated plant cells and the like thus obtained are subjected to the selection of transformants. The selection may also be based on the expression of marker genes present on the nucleic acid construct used for the transformation. For example, when the marker genes are drug resistant genes, the selection can be conducted by culturing or growing the manipulated plant cells on a culture medium containing a suitable concentration of an antibiotic or a herbicide. When the marker gene is, for example a β-glucuronidase gene or a luciferase gene and the like, the transformants can be selected by screening for the activity. From thus identified transformants such as protoplasts, calli and explants, the plant bodies can be regenerated. Known regeneration methods for each host plant may be employed for the regeneration. The plants thus obtained can be cultured by an ordinary method or, in other words, under the same conditions as those for the untransformed plants or under conditions suitable for

the respective transformants. For the identification of the transgenic plants containing the nucleic acid constructs of the present invention, various molecular biological methods can be employed in addition to the above-described marker gene selection method. Southern hybridization, PCR, Northern hybridization and RT-PCR and the like may be used to confirm the insertion of GGT gene into the genome, to identify the location of insertion, to confirm the inserted copy numbers and the like.

Then the resulting transgenic plants may be estimated for the amount of the GGT protein, the GGT activity and the amount of mRNA of GGT. For example, the amount of the protein can be determined by Western blotting method or the like, and the amount of the mRNA can be determined by Northern blotting method, quantitative RT-TCR method or the like. GGT activity can be determined by an ordinary method (Plant Physiol. 99: 1520-1525). For example, GGT activity in a photosynthetic tissue can be determined by freezing the photosynthetic tissue of a plant such as leaves with liquid nitrogen, pulverizing the frozen tissue, suspending the obtained powder in a suitable extraction buffer such as the buffer containing 100 mM Tris-HCl (pH 7.3) and 10 mM DTT, ultra-filtrating the obtained suspension, and subjecting the obtained specimen to the above-described determination method (Plant Physiol. 99: 1520-1525). GGT activity localized in the peroxisome can be determined by isolating the peroxisomes by an ordinary method (Plant Physiol. 43: 705-713, J. Biol. Chem. 243: 5179-5184, Plant Physiol. 49: 249-251 or the like) and then determining the activity by the above-described method. These methods are well known in the art.

According to the present inventions, the GGT activity of the transgenic plants increases more than about 1.2-fold, preferably more than about 3-fold, most preferably more than about 5-fold as compared with the GGT activity in the corresponding tissue of the corresponding non-transformed plants which is cultivated under the same conditions.

The resulted plants may be estimated for the amino acid content. The amino acid content can be determined by, for example, pulverizing the plant body

or a part thereof and examining the extract with a conventional amino acid analyzer. For example, amino acids can be extracted by adding 500 μl of 80 % ethanol to a sample (a plant body or a part thereof), pulverizing the sample with a cell blender MM 300 (QIAGEN) and treating the obtained product at 80°C for 10 minutes. The product is centrifuged and then subjected to vacuum concentration. The remaining sample is dissolved in 0.02 N HCl to obtain an analysis sample. The sample is passed through 0.22 μm filter to remove impurities. For the amino acid analysis, amino acid content can be determined with amino acid analyzer LS-8800 (HITACHI). The amino acids content in a plant may be quantified by using the total amount of amino acids, the amount of at least one of serine (Ser) and arginine (Arg), or increase rete of the amount of total amino acids, at least one of Ser, Arg, Gln and Asn as an indicator and is optionally processed statistically, in a particular tissue, preferably a photosynthesis tissue such as a leaf compared to the control plant grown under the same conditions. When the increase in at least one of these indices is statistically significant, it may be considered that the total amino acids content or at least one of the content of Ser, Arg, Gln, and Asn is significantly increased as compared with that of the control plant, respectively depending on the results.

A plant where the expression of the GGT gene is increased may be obtained from a plant library where an enhancer or a T-DNA tag has been randomly inserted into plants.

Furthermore, a plant where the expression of the GGT gene is increased may be obtained without using a direct molecular biological technique such as described above. Namely, a plant where the GGT gene expression is enhanced and the activity of GGT is increased can be obtained by acting a known mutagen to a plant and selecting the plant using aforementioned properties as the indicators. The substances for inducing a mutation and the methods of introducing a mutation into a plant are well known to those skilled in the art. For example, EMS, methylnitrosourea, γ-ray, ion beam, X-radiation may be used as a mutagen.

According to the present invention, a plant having increased amino acids content, particularly a plant where the content of at lease one of Ser, Arg, Gln and Asn is increased can be obtained. Specifically, According to the present invention, a mature plant can be obtained wherein the amino acids content of the plant preferably increased about 1.5-fold, more preferably about 4-fold as compared with the corresponding non-transformed plant or the wild type plant which is cultivated under the same conditions. Particularly, for Ser content, the content may increase more than about 2-fold, preferably more than about 3-fold, particularly preferably more than 20-fold as compared with the wild type plant of the same species or the corresponding non-transformed plant. Regarding Arg, Gln, Asn content, more than 1.5-fold increase, preferably more than 3-fold increase, most preferably more than 5-fold increase is achieved. Especially, more than 5-fold increase is achieved for Asn and Arg.

Additionally, Ser content can be particularly increased by cultivating the plants of the present inventions by limiting the nitrogen fertility to nitrate nitrogen. On the other hand, Asn, Gln and Arg content as well as Ser content can be increased by incorporating ammonia nitrogen in the nitrogen fertilizer. Thus, the amino acids content of the plants of the present inventions may be controlled by changing the cultivation condition, particularly by changing the nature of the nitrogen fertilizer.

Once the plant having increased amino acids content is identified, it is possible to examine whether the characteristics thereof can be stably kept genetically or not. For this purpose, plants may be cultivated under an ordinary light condition, the seeds thereof may be taken and the phenotypes and the segregation of the descendants thereof may be analyzed. For the transformants, the presence or absence of the introduced nucleic acid constructs, the position thereof and the expression thereof in the progenies may be analyzed in the same manner as that of the primary transformants. When the plants are obtained without using a direct gene introduction, the presence or absence of the mutations and their location can also be analyzed similarly.

The plants having increased amino acids content are either heterozygous or homozygous regarding the sequence derived from the nucleic acid constructs integrated into the genomes or as for the mutated or disrupted genes. If necessary, either heterozygotes or homozygotes can be obtained by, for example, cross-fertilization. The sequences derived from the nucleic acid constructs which have been integrated into the genomes segregate according to Mendel's law in the descendants. Therefore, for the object of the present invention, it is preferred to use homozygous plants from the viewpoint of the stability of the characters. The plants of the present invention can be grown under ordinary cultivation conditions.

The plants according to the present inventions may be produced and/or propagated by regenerating the plant bodies from the cells or the parts of the plants having increased GGT activity or those having increased amino acids content as described above. The plants having the features of the plants according to the present inventions may be regenerated by culturing the cells or the tissues of the plants of the present invention on a medium where MS basal medium is supplemented with appropriate hormones, optionally through the formation of embryogenesis or cell aggregation such as callus formation. These techniques for regenerating a plant body from plant cells or from parts of plants are well known to those skilled in the art. If the plants according to the present invention having increased GGT activity or having increased amino acids content as described above are capable of seed propagation, the plants according to the present inventions having aforementioned features may be obtained by collecting seeds, preferably heterozygous seeds, from the plants according to the present inventions and seeding them according to the conventional procedures, such as simply seeding them on an appropriate soil.

In the production of the seeds of the present invention, it is particularly preferred to cultivate the homozygous plants and harvest the seeds thereof. The homozygous plants may be selected by repeating the cultivation of the generations until the interested phenotypes do not segregate or, in other words, the homozygous plants can be selected by selecting the lines exhibiting the

interested phenotype in all the progenies thereof. The homozygotes can be selected by PCR or Southern analysis. By determining amino acids content of the plant by a method such as the above-described method, the seeds of the present invention may be confirmed to have amino acid content higher than the seeds of the corresponding wild-type plant cultivated under the same conditions, especially as to the content of at least one of Ser, Arg, Gln and Asn.

Additionally, if the plants according to the present invention are capable of vegetative propagation, the plants having the features of the plants of the present inventions can be directly propagated from parts of the plants. These propagation procedures are well known to those skilled in the art (For example, "Engei-Daihyakka 10 Saibai no Houhou", 1980, Koudansha may be referred to.). Such vegetative propagation procedures include, but are not limited to, the procedures using tuberous roots or tubers such as those used for potato family or carrot, those using cuttage or graftage of plants. The plants produced and/or propagated as such can be estimated for the properties, particularly for the amino acids content as described above.

The plants and seeds of the present invention are usable as foods and food materials in the same manner as the corresponding wild-type plants. Therefore, the plants and seeds of the present invention are directly usable as foods or after cooking or processing by an ordinary method, and they can be also used for feed products.

To obtain a plant extract containing amino acids, particularly at least one of Ser, Arg, Gln and Asn from the plants having increased amino acids content, particularly from the plants where at least one of Ser, Arg, Gln and Asn is increased, conventionally known methods for extracting amino acid fractions from plants, especially those for extracting fractions containing at least one of Ser, Arg, Gln and Asn can be used. For purification of any one of Ser, Arg, Gln or Asn from the extract containing at least one of these amino acids, numerous methods known to those skilled in the art can be used, including various chromatography methods.

The following Examples will further illustrate the methods for obtaining the plants of the present invention by using a model plant *Arabidopsis thaliana* and rice plants as a starting material and also illustrate the features of resulting plants and seeds. It will be apparent for those skilled in the art that the plants of the present invention, their seeds and the methods of the present invention are not limited to the particular plants, *Arabidopsis thaliana* and *Oryza sativa* (rice).

According to the disclosure of the present specification, it will be apparent for those skilled in the art that GGT gene may be used as a marker gene in the production of transgenic plant. For example, GGT gene may be used for affording the resistance against substances which may specifically inhibit GGT or affording stress-resistance to screen transgenic plants under the existence of such substances or stresses.

Examples

The cultivation of plants was all performed under the following conditions.

PNS (Mol. Gen. Genet. 204: 430-434) or MS (Physiol Plant 15: 473-479) inorganic salts containing 1 % (w/v) sucrose, 0.05 % (w/v) MES [2-(N-morpholino) ethanesulfonic acid] and 0.8 % (w/v) agar were used as the basal medium for plates. During the cultivation on rock wools, only PNS inorganic salts were used as a source of nutrient.

The GGT-knockout *Arabidopsis thaliana* strain, which had been previously obtained, was used for transformation experiments as a model plant. The method of preparing the GGT-knockout strains is shown in the following Reference Examples 1 and 2.

Reference Example 1: Preparation of GGT-knockout Arabidopsis thaliana lines

(1) Preparation of primers for screening GGT-knockout lines

Since GGT gene is also AlaAT gene, GGT gene was obtained based on the information about the alanine aminotransferase (AlaAT) gene of *Arabidopsis*

thaliana.

The copy number and the sequence of AlaAT are estimated from the data available on the Internet to prepare primers. According to the data retrieval using "Alanine aminotransferase" and "Arabidopsis" as key words, it was found that at least 4 copies of the genes, which were supposed to be alanine aminotransferase, were present on the genome. Genbank accession numbers of the respective genes were AC005292 (F26F24.16), AC011663 (F5A18.24), AC016529 (T10D10.20) and AC026479 (T13M22.3). The genes were named as GGT1, GGT 2, GGT3 and GGT4, respectively. The cDNA nucleotide sequences are shown in SEQ ID Nos:1, 3, 5 and 7, respectively, and the dedicated amino acid sequences are shown in SEQ ID Nos:2, 4, 6 and 8, respectively. The homology of GGT2, GGT3 or GGT4 against GGT1 is shown in Table 1. The comparison of the deduced amino acid sequences is shown in Figure 2.

Table 1. %Homology between GGT1 and GGT2, GGT3 or GGT4

	Homology in amino acid sequence	Homology in cDNA nucleotide sequence
GGT2	92.93	75.68
GGT3	44.71	46.72
GGT4	44.67	48.06

According to the EST information, the amount of the expression of GGT1 was supposed to be highest among the 4 copies. PCR primers for screening the gene disruption strains were prepared based on the GGT1 sequence (Table 2).

These primers were designed according to the system provided by Kazusa DNA Laboratory.

Table 2. PCR primers for screening the gene destruction strains

Name	Sequence*	
AAT1 U	CTCTAGAACCGAACGTGACTCTCCAG	(SEQ ID NO:9)
AAT1 L	CCATGATCTCCGGCATCTCATCTTC	(SEQ ID NO:10)
AAT1 L2	ATCACAAATCAGGCACAAGGTTAGAC	(SEQ ID NO:11)
AAT RTU	GGAGGGAAGAAGTGAGCTAGGGATTG	(SEQ ID NO:12)
AAT RTL	CGCTCATCCTGGTATAT GTTCTGCTG	(SEQ ID NO:13)
00 L	ATAACGCTGCGGACATCTAC	(SEQ ID NO:14)
02 L	TTAGACAAGTATCTTTCGGATGTG	(SEQ ID NO:15)
03 L	AACGCTGCGGACATCTACATTTTTG	(SEQ ID NO:16)
04 L	GTGGGTTAATTAAGAATTCAGTACATTAAA	(SEQ ID NO:17)
05 L	AAGAAAATGCCGATACTTCATTGGC	(SEQ ID NO:18)
06 L	AAGAAAATGCCGATACTTCATTGGC	(SEQ ID NO:19)
00 R	TAGATCCGAAACTATCAGTG	(SEQ ID NO:20)
02 R	ACGTGACTCCCTTTAATTCTCCGCTC	(SEQ ID NO:21)
03 R	CCTAACTTTTGGTGTGATGATGCTG	(SEQ ID NO:22)
04 R	TTCCCTAAATAATTCTCCGCTCATGATC	(SEQ ID NO:23)
05 R	TTCCCTTAATTCTCCGCTCATGATC	(SEQ ID NO:24)
06 R	TTCCCTTAATTCTCCGCTCATGATC	(SEQ ID NO:25)
EF U	GTTTCACATCAACATTGTGGTCATTGG	(SEQ ID NO:26)
EF L	GAGTACTTGGGGGTAGTGGCATCC	(SEQ ID NO:27)

* The sequences are shown in the direction of 5' -> 3' according to the conventional notation.

(3) Isolation of GGT destruction strains

The screening for GGT in the gene disruption *Arabidopsis thaliana* Library was performed using the system provided by Kazusa DNA Research Institutes. The screening was conducted by the procedure described in 2-4-c in Plant Cell Engineering Series 14 "*Shokubutsu no Genome Kenkyu Protocol* (Protocol of Study of Plant Genome)" (Shujunsha).

In the primary screening, (AAT1U/AAT1L) was used as the primer for the gene, and (00L/02L/03L/04L/05L/06L/00R/02R/03R/04R/05R/06R) were used as the tag primers for the respective corresponding pools. The relationship among the tag primers used and the respective pools are shown in Table 3.

Table 3. Relationship between tag primers and pools

DNA pool			Number of pools	Tag primer	
P0009	~	P0020	12	00R	00L
P0023	~	P0040	18		
P0202	~	P0204	3	02R	02L
P0301	~	P0302	2	03R	03L
P0401	~	P0403	3	04R	04L
P0501	~	P0508	8	05R	05L
P0601	~	P0608	8	06R	06L
		Total	54		

The polymerase used was EX-taq (TAKARA). 20 μl of the reaction solution contained about 38.4 ng (about 100 pg x 384) of template DNA, 10 pmol of tag primer, 10 pmol of primer for the gene, 2 μl of 10 x buffer, 5 nmol of dNTPs and 0.5 U of Ex-taq. PCR was conducted by 35 cycles of 94°C for 45 seconds, 52°C for 45 seconds and 72°C for 3 minutes. Then, 10 μl of the PCR product was resolved by electrophoresis on 1 % agarose gel. The amplified DNA fragments were observed after EtBr staining. The gel was denatured by the immersion in a denaturing solution (1.5 M NaCl, 0.5 M NaOH) for 20 minutes. The gel was then immersed in a neutralizing solution [0.5 M Tris-HCl (pH 8.0), 1.5 M NaCl] for 20 minutes. After blotting onto membrane-Hybond N+ (Amersham Pharmacia Biotech) with 20 x SSC (3M NaCl, 0.3 M sodium citrate), DNA was fixed on the membrane by UV cross-linking. The hybridization and detection were conducted with AlkPhos-Direct DNA detection kit (Amersham Pharmacia Biotech) according to the protocol attached thereto. The hybridization temperature was 65°C. PCR was conducted using AATIU/AAT1L and genome DNA as a template. The amplified fragments were purified with GFX PCR DNA and Gel Band purification kit (Amersham Pharmacia Biotech).

In the primary screening, a mixture of genome DNA extracted from 384 independent tag-inserted strains was taken as one pool. 54 pools (384 x 54 = 20736 lines) were subjected to PCR. The amplification products were subjected to Southern analysis to confirm whether the intended product was amplified or not.

Pool P0035 having positive results in the primary screening was subjected to

the secondary screening. The primer combination for PCR for the secondary screening was AAT1U/00L and AAT1L/00L, which gave positive results in the primary screening. By the secondary screening, it was revealed that GGT1 tag was inserted in one line, line 8046.

(4) Determination of the location of tag insertion

DNA extracted from the determined tag-inserted line was used as a template. PCR was conducted by using two primer sets (AAT1U/00L, AAT1L/OOL). The amplified fragments were cloned to obtain pGEM T-easy vector (Promega). DNA sequencer, ABI PRISMTM 377 DNA sequencer (PERKIN ELMER) was used for sequencing.

It was found that the tag was inserted in the sixth exon with the deletion of 16bp and that 176-GGTLV-180 was replaced with 176-AIQL (end)-180 by the insertion of the tag.

Reference Example 2: Preparation of GGT-knockout homozygotes

(1) Selection of homozygotes

T2 seeds of the line of which the tag insertion had been confirmed were placed on MS medium containing 10 mg/l of hygromycin. Three weeks later, they were transplanted on rock wools, and DNA was extracted from about 5 mm x 5 mm samples of rosette leaves. The extraction was conducted according to Li method (Plant J. 8: 457 to 463). For the identification of the homozygotes, PCR was conducted with the primers (AAT1U/AAT1L2) flanking the tag. PCR was conducted by 30 cycles of 94°C for 30 seconds for denature, 57°C for 30 seconds for annealing and 72°C for 60 seconds for elongation. For the control, wild type genome DNA was used as the template. An aliquot of the PCR product was resolved on 1 % agarose gel by electrophoresis. In total 35 lines, eleven (11) lines were found to be homozygotes.

(2) Detection of GGT expression

The obtained homozygous lines were subjected to RT-PCR by using the progenies thereof to confirm that the gene disruption occurred. The seeds of the homozygotes were seeded on MS medium containing 10 mg/l of hygromycin, and it was confirmed that all the individuals exhibited the resistance. Total RNA was extracted from seedlings with ISOGEN (Nippon gene) two weeks after seeding the seeds. After the treatment with DNase followed by the reverse transcription with oligo-dT primer using superscript II (GIBCO), PCR was conducted with the primers (AAT1 RTU / AAT1RTL) flanking the tag using the synthesized single-strand cDNA as a template. 28 cycles of PCR were conducted, wherein denaturation was conducted at 94°C for 30 seconds, the annealing was conducted at 57°C for 30 seconds and the elongation was conducted at 72°C for 60 seconds. For the control, EF1-α (EFU/EFL) was used. An aliquot of the PCR product was resolved on 1 % agarose gel by electrophoresis. No full-length mRNA for GGT1 was found in the tag-inserted lines.

According to these results, the tag-inserted strain was named "ggt1-1" and used for the following analysis. The growth of ggt1-1 strain was significantly inhibited under the ordinary light strength condition, but no significant difference was found as to the growth under the weak light condition (about 30μmol m^{-2} s^{-1}) as compared with the non-transformed plant. Additionally, it was found that the GGT activity was remarkably reduced in ggt1-1 as measured by the method described hereinafter. Therefore, ggt1-1 was used as the experimental material for increasing GGT activity.

Example 1: Generation of transgenic plants having increased GGT activity

(1) Introduction of the genetic construct for GGT gene expression

The 5089bp genome region of GGT1 was amplified by PCR procedure. The upstream primer was 5'- CAATAACAATGCAAAGTTAAGATTCGGATC -3' (SEQ ID NO:28) and the downstream primer was 5'- GCTTCTTCTCAACCATCGTCACC -3' (SEQ ID NO: 29). The nucleotide sequence encoding GGT1 and the amino acid sequence of GGT was show in SEQ ID NOs:1

and 2, and the construct of the introduced gene was shown in Figure 3. The amplified fragment was inserted in the HindIII and BamHI site of binary vector pBI101 having its Gus/Nos-ter deleted and the cloned fragment was introduced into GGT1 gene-knockout *Arabidopsis thaliana* strain (ggt1-1). The resulted transformants were plated on PNS medium and were grown for 2 weeks under a light condition of 70μmol m^{-2} s^{-1}. After that, the weight of the aboveground part of the seedlings was determined. The results showed that the growth inhibition caused by the gene disruption was completely complemented and furthermore the growth was enhanced as compared with the wild type (Figure 4).

(2) Confirmation of GGT gene expression

The expression of the introduced gene was confirmed by quantitative PCR. The seeds were plated on a 1/2 MS medium containing 50mg/ml kanamycin and the lines of which individuals exhibited the resistance were selected as a source of RNA. Total RNA was extracted from the aboveground parts of the seedlings that were grown on PNS medium for 2 weeks under the light condition of 30μmol m^{-2} s^{-1} by using RNeasy Plant Mini Kit (QIAGEN). After DNase treatment, reverse transcription was conducted starting from oligo dT primer by using superscriptII (GIBCO) to synthesize a single strand cDNA which was in turn used as a template for PCR with the quantitative PCR primer (5'-TTCTTCTTCTGAACGACTATTGTG -3' : SEQ ID NO:30 and 5'-GAATAGGGCAAAGAGAAAGAGTG -3' : SEQ ID NO:31).

The primers 5'- GGTAACATTGTGCTCAGTGGTGG -3' : SEQ ID NO:32 and 5'- GGTGCAACGACCTTAATCTTCAT -3' : SEQ ID NO:33 were used for the quantitative PCR of ACTIN2. The quantitative PCR was conducted by ABI PRISM 7700 with the following condition: 1 cycle of 50 °C for 2 minutes and 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds.

RNA was extracted and tested in triplicate for the independent experiments and the expression of GGT1 was normalized by the expression level

of ACTIN2. The quantification of GGT1 expression level was shown in Figure 5. The expression level was increased about 2-fold in the transgenic line.

Example 2. Evaluation of the features of transgenic plants having enhanced GGT activity

(1) Determination of GGT enzyme activity

For determining the enzymatic activity, proteins were extracted from seedlings grown under a light condition of 70μmol m^{-2} s^{-1} for 2 weeks after plating on PNS medium. The plant (fresh weight: about 200 mg) was frozen in liquid nitrogen and then the tissues thereof were crushed by using a mortar and a pestle. 1 ml of the extraction buffer [100 mM Tris-HCl (pH 7.3), 10 mM DTT] was added thereto, and the obtained mixture was centrifuged at 15,000 rpm for 10 minutes to remove insoluble matters. This process was repeated 3 more times. The demineralization was conducted with a ultrafiltration filter UFV5BGCOO (Millipore). 0.5 ml of the extract was concentrated to a concentration of 10 times by centrifuging it at 10,000 rpm for about 45 minutes. After diluting the extract by 10-fold, the same process was repeated 3 times. The protein concentration was determined with a protein assay kit (Bio-Rad). The extraction buffer containing 10 % glycerol was added thereto to obtain a final concentration of 1mg/ml of extract to obtain the crude extract.

The activity of GGT (Glu + glyoxylate -> Gly + αKG) was determined as the change in OD at 340 nm by coupling the reaction with the oxidation reaction of NADH by NAD^+-GDH (EC 1.4.1.3). The reaction was conducted by using 50 μg of the crude extract in 0.6ml of the reaction solution [100 mM Tris-HCl (pH 7.3), 100 mM Glu, 0.11 mM pyridoxal 5-phosphate, 0.18 mM NADH, 15 mM glyoxylate, 500 U/l GDH (G2501)]. The activity of HPR was used for the control. The activity of HPR was determined by the change in OD at 340nm due to the oxidation of NADH. The reaction was conducted by using 50μg crude extract in 0.6ml of reaction solution [100mM Tris-HCl (pH7.3), 5mM hydroxy pyruvate and 0.18mM NADH]. The activities of GGT and HPR were shown in Figure 6. The

GGT activity was found to be about 2-fold higher in the GGT transgenic lines than the corresponding non-transformed plants.

(2) Analysis of amino acids

For determining free amino acids content, amino acids were extracted from the seedlings grown under the light condition of 70μmol m^{-2} s^{-1} for 2 weeks after plating on PNS medium and also from the rosette leaf of the plants grown for 6 weeks on a rock wool using PNS as a nutrient. The plant (fresh weight: about 100 mg) was frozen in liquid nitrogen and then stored at -80°C. To the frozen sample, 500 μl of 80 % ethanol was added, the tissue was crushed with a cell crusher MM 300 (QIAGEN) and then treated at 80°C for 10 minutes to extract amino acids. After the centrifugation at 15,000 rpm for 10 minutes, the supernatant was removed, and 500 μl of 80 % ethanol was added at 80°C to the obtained precipitate, and the mixture was thoroughly stirred and then treated at 80 ℃ for further 10 minutes. After the centrifugation at 15,000 rpm for 10 minutes, the supernatant was taken as the amino acids extract. 1 ml of the amino acid extract was rotated under reduced pressure to completely remove ethanol and water. The sample was dissolved in 500ml of water and the equivalent volume of diethyl ether. The lower layer obtained after centrifugation was rotated under reduced pressure. 0.02 N HCl was added to the remaining sample to a final concentration of 10μl/mg FW and Vortexed followed by centrifugation to recover the supernatant. The impurities were removed by passing the supernatant through a 0.22μm filter to obtain the sample for analysis.

The amino acid analysis was conducted with an amino acid analyzer LS-8800 (HITACHI). The total amino acids content and major amino acids content (nmol/mg FW) were shown in Figures 7 and 8. The results showed that serine content was remarkably increased in the GGT1 overexpressing lines and the total amino acids content and the arginine content were increased in the plants grown on rock wools.

(3) Analysis of nitrogen content

The nitrogen content was determined in the aboveground parts of the seedlings grown under the light condition of 70μmol m^{-2} s^{-1} for 2 weeks after seeding on PNS medium. The determination was performed by using Sumigraph NC-1000 manufactured by Sumitomo Chemical Analysis Center. The results showed the nitrogen content per dry weight was increased in the GGT overexpressing strains, as indicated in Table 4.

Table 4. %Ratio of total nitrogen per dry weight

ggt1-1/GGT1 strain	7.21
Control plant (wild type non-transformed plant)	7.10

Example 3: Generation of transgenic plants having much more increased GGT activity

To generate a plant having much more increased GGT activity, a genetic construct for expressing GGT gene was introduced into the wild type plant and the property of the plant was evaluated. The GGT1-transgenic strain obtained by introducing a GGT expressing construct into GGT1 gene disrupted strain (ggt1-1) is hereinafter referred to as "ggt1-1/GGT1" strain and the GGT1-transgenic strain obtained by introducing a GGT expressing construct into the wild type plant is hereinafter referred to as "WT/GGT1" strain.

(1) Introduction of the genetic construct for GGT gene expression

GGT1 gene was introduced to the wild type (Col-0) by using the similar procedures described in Example 1 (1).

(2) Confirmation of the expression of GGT gene

The expression of the transgene was confirmed by the method described in Example 1 (2). For the source of RNA, the wild type strain grown for 2 weeks on PNS medium, 2 lines from ggt1-1/GGT1 and seven lines from WT/GGT1 were used. The quantification of GGT1 expression was shown in Figure 9. The

expression level was increased 5- to 30-fold in the transgenic lines.

Example 4: Evaluation of the transgenic plants having much more enhanced GGT activity

(1) Determination of GGT enzyme activity

The determination of the enzyme activity was conducted by the methods described in Example 2 (1). GGT activity and the control HPR activity were shown in Figure 10. The GGT activity was increased about 2- to 6-fold in the GGT transgenic lines as compared with the wild type.

(2) Amino acid analysis

The contents of the free amino acids were determined by the method described in Example 2 (2). The serine content (nmol/mg FW) of the strains of which GGT expression level and the enzyme activities were determined in Example 3(2) and Example 4 (1) were shown in Figure 11 for PNS medium cultivation. The determined results obtained from 40 lines in total were shown in Table 5. The relationships between expression level, enzyme activities and serine content were shown in Figure 12. The contents of major amino acids and the total amino acids of the plants grown on 1/2 MS medium were shown in Figure 13. The amino acid contents of seeds were shown in Figures 14 and 15. The results of the analysis showed that the serine content increased up to 20-fold in the GGT1 overexpressing lines. The comparison of expression levels, enzyme activities and serine contents revealed that they had a significant relationship each other.

Table 5. Ser Content

Line	Ser Content (nmol/mgFW)
Control	0.69
WT/GGT1 No. 1	7.43
WT/GGT1 No. 2	2.14
WT/GGT1 No. 3	7.33
WT/GGT1 No. 4	8.42

WT/GGT1 No. 5	7.68
WT/GGT1 No. 6	10.07
WT/GGT1 No. 7	5.84
WT/GGT1 No. 8	4.54
WT/GGT1 No. 9	10.13
WT/GGT1 No.10	8.51
WT/GGT1 No.11	3.03
WT/GGT1 No.12	8.01
WT/GGT1 No.13	4.84
WT/GGT1 No.14	3.07
WT/GGT1 No.15	6.97
WT/GGT1 No.16	6.92
WT/GGT1 No.17	5.44
WT/GGT1 No.18	7.41
WT/GGT1 No.19	9.06
WT/GGT1 No.20	4.01

Table 5. (Continued)

Line	Ser Content (nmol/mgFW)
WT/GGT1 No.21	8.20
WT/GGT1 No.22	3.20
WT/GGT1 No.23	5.92
WT/GGT1 No.24	6.53
WT/GGT1 No.25	5.42
WT/GGT1 No.26	8.66
WT/GGT1 No.27	1.48
WT/GGT1 No.28	7.37
WT/GGT1 No.29	7.32
WT/GGT1 No.30	11.66
WT/GGT1 No.31	8.06
WT/GGT1 No.32	8.91
WT/GGT1 No.33	8.19
WT/GGT1 No.34	14.25
WT/GGT1 No.35	12.80
WT/GGT1 No.36	11.89
WT/GGT1 No.37	11.28
WT/GGT1 No.38	7.01
WT/GGT1 No.39	5.01
WT/GGT1 No.40	3.43

When the plants were grown on 1/2 MS medium, asparagine increased about 5-fold, glutamine increased about 3-fold, arginine increased about 5-fold and the total amino acids increased about 4-fold in Strain No.4 as compared with the

wild type. Furthermore, in the GGT1 overexpression lines, Ser content was remarkably increased when the lines were grown on PNS medium and besides Ser, asparagine, glutamine and arginine were increased about 3- to 5-fold when the lines were grown on 1/2 MS medium containing ammonia-nitrogen.

The amino acids in the seeds were determined in the seeds from the plants grown under the light condition of about 200μmol m^{-2} s^{-1} continuous light on the modified PNS (5mM KNO_3 was replaced with 2.5mM NH_4NO_3). Asparagine, aspartate, glutamate, serine, glycine and arginine were accumulated and increased in ggt1-1/GGT1 No. 4-7 line as compared with the wild type. The total amino acids were also increased.

Example 5: Generation of tomato GGT transformants and potato GGT transformants

(1) Generation of tomato transformants

Seeds of tomato (cultivar, Mini-tomato Fukukaenn-Shubyou) are surface-sterilized by 70% ethanol (30 seconds) and 2% sodium hypochloride (15 minutes), placed on plant hormone-free MS-agar plates and grown at 25°C for 1 week under 16-hour daylight. The cotyledons are picked up from the resulting sterile seedlings and placed on MS agar plates containing 2mg/ml zeatin and 0.1mg/ml indoleacetate (regeneration medium, 9cm dish) and further cultivated for 2 days under said condition. The Agrobacterium (EHA101) harboring the constructed gene are grown in YEP medium (Table 6) overnight and used for infection. The cotyledons that have been cultured for 2 days are collected in a dish and the Agrobacterium suspension was added for infection. Sterile filter is used for removing the Agrobacterium suspension from the cotyledons and the infected cotyledons were placed on a sterile filter which is placed on the aforementioned medium plate to avoid the rapid growth of the agrobacteria. The cotyledons are co-cultured for 24 hours.

After the period, the cotyledons are transferred onto a MS regeneration medium (selection medium) containing 50mg/ml kanamycin and 500mg/ml

Claforan to select the transformants. The regenerated shoots are transferred to a fresh selection medium for re-selection. The vigorously growing green shoots are cut at the stems and placed on the MS medium (rooting medium, in tubes) which is free of plant hormones. The rooted regenerated plants are continuously acclimated to soils.

Table 6. YEP medium composition

YEP medium ingredients	(1 liter)
Bacto Trypton	10 g
Yeast Extract	10 g
Glucose	1 g

(2) Generation of potato transformants

The sterile potato plants were obtained by stem apex culture and the materials were increased by subculturing the stem apexes. The stem apexes were induced for rooting by placing them into MS liquid medium (10ml) supplemented with 2% sucrose. After rooting, 10 ml of MS liquid medium containing 16% sucrose was added and the stem apexes were culture under the dark place to induce microtubers. The microtubers of 6-8 weeks culture are sectioned into discs, peal and are infected with agrobacteria into which the genetic construct described in Example 1 (1) has been introduced and which has been grown overnight at 28 °C. The discs are placed on a sterile filter which is laid on a MS agar plate (MS medium, 2.0mg/ml zeatin, 0,1mg/l indoleacetate, 0.3% gelite) and are co-cultured for 2 days at 25°C under 16-hours daylight. Then the discs are transferred to a selection medium [Ms medium, 2.0mg/ml zeatin, 0,1mg/l indole acetate, 0.3% gelite, 50mg/l kanamycin, 500mg/l Claforan] and cultur under the same condition. They are transferred onto a fresh selection medium every one week and the regenerated shoots are transferred to a selection medium which do not contains plant hormones to induce rooting. They are infected with the agrobacteria into which the genetic construct described in Example 1 (1) has been introduced and are selected on a medium containing 50mg/ml of kanamycin.

Example 6: Generation of rice GGT transformants

(1) Generation of *Arabidopsis thaliana* GGT1 gene introduced rice

The cDNA of *Arabidopsis thaliana* GGT1 gene region was amplified by PCR method. The primer 5'- GCGGATCCATGGCTCTCAAGGCATTAGACT -3': SEQ ID NO:38 was used for the upstream primer and 5'- GCCGAGCTCTCACATTTTCGAATAA -3': SEQ ID NO:39 was used for the downstream primer. The amplified fragment was linked to the downstream of CAB promoter (Plant Cell Physiol 42, 138-, 2001) using the underlined restriction enzyme site (BamHI, SacI) to replace the 35S promoter + GUS region in the binary vector pIG121HM. This was introduced into a rice plant (race = Kiatake) through Agrobacterium. The transformation was conducted according to the Method of Toriyama et al. (Experimental Protocols for Model Plants, 93-, 1996, Shujunsha).

The individual plants that exhibited the resistance on a selection medium containing hygromycin were transferred onto soils and the leaves were sampled for RNA extraction and amino acid analysis.

(2) Confirmation of GGT1 gene expression

The expression of the transgene was confirmed by RT-PCR for 20 strains that had been selected for the drug resistance. Total RNA was extracted by using RNeasy Plant Mini Kit (QIAGEN). After DNase treatment, reverse transcription was conducted with oligo dT primer using superscript II (GIBCO) and the synthesized single strand cDNA was used as a template for PCR using PCR primers (5'- TGAAAGCAAGGGGATTCTTG -3': SEQ ID NO:40 and 5'- GACGTTTTTGCAGCTGTTGA -3': SEQ ID NO: 41). The reaction was performed under the condition of 40 cycles of 95°C for 15 seconds, 60°C for 60 seconds. The amplification of GGT1 DNA fragment was confirmed in the tested 20 lines of transformant, which was indicative of the expression of the transgene.

(3) Amino acid content of the GGT1 transgenic rice

The determination of free amino acids content was performed according to the method described in Example 2 (2). It was shown that Ser was significantly increased in the transformants as compared with the non-transformants (Figure 17).

<Sequence listing free text>

SEQ ID NOs:9-33, 38-41: PCR primer

According to the present invention, a novel method for utilizing glutamate glyoxylate amino transferase (GGT) for improving the properties of plants.

According to the present invention, a plant having increased GGT activity is provided. Particularly, according to the present invention a plant having increased GGT activity preferably more than about 2-fold, more preferably more than about 3-fold, and most preferably more than about 5-fold.

Additionally, according to the present invention, a method of increasing the amino acids content in a plant and/or a seed, particularly a method of increasing at least one of Ser, Arg, Gln and Asn, a plant and/or a seed having increased amino acids contents, particularly a plant and/or a seed where at least one of Ser, Arg, Gln and Asn content is increased, the use of such plants and/or seeds for the production of feeds and a feed containing a plant and/or a seed having increased glutamate content.

A plant extract containing at least one of the amino acid Ser, Arg, Gln and Asn in a large amount can be easily obtained according to the present invention.

Furthermore, it has been suggested that there is a strong correlation between the lysine content and the content of glutamine, glutamate, asparagine and aspartate (Plant Cell 15, 845-853, 2003). Therefore, the plant of the present invention or the method of producing such plants may also provide a plant having increased lysine content.

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ctt gac tcc atc aat ccc aag gtt ctg aaa tgt gag tat gct gtt cga 240
Leu Asp Ser Ile Asn Pro Lys Val Leu Lys Cys Glu Tyr Ala Val Arg
65 70 75 80

gga gaa att gtc aac att gct cag aag tta caa gaa gac ttg aag act 288
Gly Glu Ile Val Asn Ile Ala Gln Lys Leu Gln Glu Asp Leu Lys Thr
85 90 95

aat aag gat gct tat ccc ttt gat gag ata atc tat tgc aac att ggg 336
Asn Lys Asp Ala Tyr Pro Phe Asp Glu Ile Ile Tyr Cys Asn Ile Gly
100 105 110

aat cct caa tct ctt ggt cag ctg cct ata aag ttc ttc cgt gag gtt 384
Asn Pro Gln Ser Leu Gly Gln Leu Pro Ile Lys Phe Phe Arg Glu Val
115 120 125

ctc gca ttg tgt gac cac gca agt ctt ttg gat gag tct gaa acc cat 432
Leu Ala Leu Cys Asp His Ala Ser Leu Leu Asp Glu Ser Glu Thr His
130 135 140

ggt ttg ttc agt acc gat tca att gac cga gca tgg agg att ttg gac 480
Gly Leu Phe Ser Thr Asp Ser Ile Asp Arg Ala Trp Arg Ile Leu Asp
145 150 155 160

cat att ccc gga aga gca act ggg gct tac agt cat agc cag ggt atc 528
His Ile Pro Gly Arg Ala Thr Gly Ala Tyr Ser His Ser Gln Gly Ile
165 170 175

aag ggt tta cgt gat gta att gca gct gga atc gaa gca cgt gat ggt 576
Lys Gly Leu Arg Asp Val Ile Ala Ala Gly Ile Glu Ala Arg Asp Gly
180 185 190

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ttc cct gct gat cca aat gat att ttc ttg act gat ggt gca agt cca      624
Phe Pro Ala Asp Pro Asn Asp Ile Phe Leu Thr Asp Gly Ala Ser Pro
        195                 200                 205

gcg gtt cac atg atg atg caa ctt ctc ttg agc tca gag aaa gat ggt      672
Ala Val His Met Met Met Gln Leu Leu Leu Ser Ser Glu Lys Asp Gly
    210                 215                 220

att ctt tcc ccg att cct cag tat cca ttg tac tcg gct tca att gcc      720
Ile Leu Ser Pro Ile Pro Gln Tyr Pro Leu Tyr Ser Ala Ser Ile Ala
225                 230                 235                 240

ctt cat ggt gga tct ttg gtt ccg tac tat ctt gat gaa gca aca gga      768
Leu His Gly Gly Ser Leu Val Pro Tyr Tyr Leu Asp Glu Ala Thr Gly
                245                 250                 255

tgg gga ctg gaa ata tct gac ctt aag aag caa cta gag gaa gct agg      816
Trp Gly Leu Glu Ile Ser Asp Leu Lys Lys Gln Leu Glu Glu Ala Arg
            260                 265                 270

tcc aag ggc atc tct gta agg gcc ttg gtt gtc ata aac ccc ggt aac      864
Ser Lys Gly Ile Ser Val Arg Ala Leu Val Val Ile Asn Pro Gly Asn
        275                 280                 285

cca act ggg cag gtt ctt gcg gaa gaa aac cag cgt gac att gtt aat      912
Pro Thr Gly Gln Val Leu Ala Glu Glu Asn Gln Arg Asp Ile Val Asn
    290                 295                 300

ttc tgc aag caa gag ggt ttg gtt ctt tta gct gat gaa gtc tac caa      960
Phe Cys Lys Gln Glu Gly Leu Val Leu Leu Ala Asp Glu Val Tyr Gln
305                 310                 315                 320

gaa aat gtt tac gtc cct gac aaa aag ttc cat tct ttc aag aaa gtg     1008
Glu Asn Val Tyr Val Pro Asp Lys Lys Phe His Ser Phe Lys Lys Val
                325                 330                 335

gct cgg tct ttg ggc tat gga gaa aaa gat atc tcg tta gtc tcg ttc     1056
Ala Arg Ser Leu Gly Tyr Gly Glu Lys Asp Ile Ser Leu Val Ser Phe
            340                 345                 350

caa tca gtc tcc aaa gga tat tat gga gaa tgt gga aaa aga gga ggt     1104
Gln Ser Val Ser Lys Gly Tyr Tyr Gly Glu Cys Gly Lys Arg Gly Gly
        355                 360                 365

tac atg gag gtt act gga ttc act tct gat gta aga gaa cag ata tac     1152
Tyr Met Glu Val Thr Gly Phe Thr Ser Asp Val Arg Glu Gln Ile Tyr
    370                 375                 380

aaa atg gct tct gtg aat ctt tgt tct aac atc tct ggt caa att ctt     1200
Lys Met Ala Ser Val Asn Leu Cys Ser Asn Ile Ser Gly Gln Ile Leu
385                 390                 395                 400

gct agc ctt gtc atg agc cca ccc aag cct ggt gat gac tca tat gac     1248
Ala Ser Leu Val Met Ser Pro Pro Lys Pro Gly Asp Asp Ser Tyr Asp
                405                 410                 415
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tca tac atg gca gaa aga gat gga att ctc tca tcc atg gct aaa cgt      1296
Ser Tyr Met Ala Glu Arg Asp Gly Ile Leu Ser Ser Met Ala Lys Arg
            420                 425                 430

gca aag act ttg gaa gac gct ctc aac agt tta gaa ggt gtt aca tgt      1344
Ala Lys Thr Leu Glu Asp Ala Leu Asn Ser Leu Glu Gly Val Thr Cys
        435                 440                 445

aac aga gcc gaa gga gca atg tat ctc ttc ccg cga att aac ctt cct      1392
Asn Arg Ala Glu Gly Ala Met Tyr Leu Phe Pro Arg Ile Asn Leu Pro
    450                 455                 460

caa aag gct atc gaa gct gct gag gct gaa aaa act gca cca gat gcg      1440
Gln Lys Ala Ile Glu Ala Ala Glu Ala Glu Lys Thr Ala Pro Asp Ala
465                 470                 475                 480

ttc tac tgc aaa cgc ctt ctc aat gct act ggt gta gtt gta gtc cct      1488
Phe Tyr Cys Lys Arg Leu Leu Asn Ala Thr Gly Val Val Val Val Pro
                485                 490                 495

ggt tct ggc ttt gga cag gtt cct gga aca tgg cac ttt aga tgc aca      1536
Gly Ser Gly Phe Gly Gln Val Pro Gly Thr Trp His Phe Arg Cys Thr
            500                 505                 510

ata ctt cca caa gaa gac aag att cca gcg ata gtg aat cgt ctg aca      1584
Ile Leu Pro Gln Glu Asp Lys Ile Pro Ala Ile Val Asn Arg Leu Thr
        515                 520                 525

gag ttc cac aag agc ttc atg gac gag ttc cgc aac taa                  1623
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<213> Arabidopsis thaliana

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Arg Pro Leu Ala Ser Ser Pro Pro Ala Leu Ser Arg Phe Phe Ser Ser
        35                  40                  45

Thr Ser Glu Met Ser Ala Ser Asp Ser Thr Ser Ser Leu Pro Val Thr
    50                  55                  60

Leu Asp Ser Ile Asn Pro Lys Val Leu Lys Cys Glu Tyr Ala Val Arg
65                  70                  75                  80

Gly Glu Ile Val Asn Ile Ala Gln Lys Leu Gln Glu Asp Leu Lys Thr
                85                  90                  95
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Asn Lys Asp Ala Tyr Pro Phe Asp Glu Ile Ile Tyr Cys Asn Ile Gly
100 105 110

Asn Pro Gln Ser Leu Gly Gln Leu Pro Ile Lys Phe Phe Arg Glu Val
115 120 125

Leu Ala Leu Cys Asp His Ala Ser Leu Leu Asp Glu Ser Glu Thr His
130 135 140

Gly Leu Phe Ser Thr Asp Ser Ile Asp Arg Ala Trp Arg Ile Leu Asp
145 150 155 160

His Ile Pro Gly Arg Ala Thr Gly Ala Tyr Ser His Ser Gln Gly Ile
165 170 175

Lys Gly Leu Arg Asp Val Ile Ala Ala Gly Ile Glu Ala Arg Asp Gly
180 185 190

Phe Pro Ala Asp Pro Asn Asp Ile Phe Leu Thr Asp Gly Ala Ser Pro
195 200 205

Ala Val His Met Met Met Gln Leu Leu Leu Ser Ser Glu Lys Asp Gly
210 215 220

Ile Leu Ser Pro Ile Pro Gln Tyr Pro Leu Tyr Ser Ala Ser Ile Ala
225 230 235 240

Leu His Gly Gly Ser Leu Val Pro Tyr Tyr Leu Asp Glu Ala Thr Gly
245 250 255

Trp Gly Leu Glu Ile Ser Asp Leu Lys Lys Gln Leu Glu Glu Ala Arg
260 265 270

Ser Lys Gly Ile Ser Val Arg Ala Leu Val Val Ile Asn Pro Gly Asn
275 280 285

Pro Thr Gly Gln Val Leu Ala Glu Glu Asn Gln Arg Asp Ile Val Asn
290 295 300

Phe Cys Lys Gln Glu Gly Leu Val Leu Leu Ala Asp Glu Val Tyr Gln
305 310 315 320

Glu Asn Val Tyr Val Pro Asp Lys Lys Phe His Ser Phe Lys Lys Val
325 330 335

Ala Arg Ser Leu Gly Tyr Gly Glu Lys Asp Ile Ser Leu Val Ser Phe
340 345 350

Gln Ser Val Ser Lys Gly Tyr Tyr Gly Glu Cys Gly Lys Arg Gly Gly
355 360 365

Tyr Met Glu Val Thr Gly Phe Thr Ser Asp Val Arg Glu Gln Ile Tyr
370 375 380

Lys Met Ala Ser Val Asn Leu Cys Ser Asn Ile Ser Gly Gln Ile Leu
385 390 395 400

Ala Ser Leu Val Met Ser Pro Pro Lys Pro Gly Asp Asp Ser Tyr Asp
405 410 415

Ser Tyr Met Ala Glu Arg Asp Gly Ile Leu Ser Ser Met Ala Lys Arg
420 425 430

Ala Lys Thr Leu Glu Asp Ala Leu Asn Ser Leu Glu Gly Val Thr Cys
435 440 445

Asn Arg Ala Glu Gly Ala Met Tyr Leu Phe Pro Arg Ile Asn Leu Pro
450 455 460

Gln Lys Ala Ile Glu Ala Ala Glu Ala Glu Lys Thr Ala Pro Asp Ala
465 470 475 480

Phe Tyr Cys Lys Arg Leu Leu Asn Ala Thr Gly Val Val Val Val Pro
485 490 495

Gly Ser Gly Phe Gly Gln Val Pro Gly Thr Trp His Phe Arg Cys Thr
500 505 510

Ile Leu Pro Gln Glu Asp Lys Ile Pro Ala Ile Val Asn Arg Leu Thr
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1 5 10 15

cgt cgt cgt caa ctt cat cat cac aaa aat ctc agc ttt gtc tct ctt 96
Arg Arg Arg Gln Leu His His His Lys Asn Leu Ser Phe Val Ser Leu
20 25 30

att cct cct ttt tct gct cct tcc gat tct tca tcg cgc cac ttg tct 144
Ile Pro Pro Phe Ser Ala Pro Ser Asp Ser Ser Ser Arg His Leu Ser
35 40 45

tct tct tct tct tcc gat atg tct gct tct gat tcc tct tcc tct ctt 192
Ser Ser Ser Ser Ser Asp Met Ser Ala Ser Asp Ser Ser Ser Ser Leu
50 55 60

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ccc gtt act ctt gac acc atc aac ccc aag gtt atc aaa tgt gag tat      240
Pro Val Thr Leu Asp Thr Ile Asn Pro Lys Val Ile Lys Cys Glu Tyr
65                  70                  75                  80

gct gtc cgt gga gaa att gtc aac att gct cag aaa ttg caa gaa gat      288
Ala Val Arg Gly Glu Ile Val Asn Ile Ala Gln Lys Leu Gln Glu Asp
                85                  90                  95

ttg aag act aac aag gac gct tat ccc ttt gat gag att atc tac tgt      336
Leu Lys Thr Asn Lys Asp Ala Tyr Pro Phe Asp Glu Ile Ile Tyr Cys
            100                 105                 110

aat atc ggg aat cct caa tct ctt ggt caa cag cct ata aca ttc ttc      384
Asn Ile Gly Asn Pro Gln Ser Leu Gly Gln Gln Pro Ile Thr Phe Phe
        115                 120                 125

aga gag gtt ctt gct tta tgt tcc tac aca gcc ctg ttg gat gag agt      432
Arg Glu Val Leu Ala Leu Cys Ser Tyr Thr Ala Leu Leu Asp Glu Ser
    130                 135                 140

gca aca cac ggt ttg ttc agg ttc agt tct gat tcg att gag cgt gct      480
Ala Thr His Gly Leu Phe Arg Phe Ser Ser Asp Ser Ile Glu Arg Ala
145                 150                 155                 160

tgg aag att ctg gac caa att ccc ggg aga gcg act ggt gct tac agc      528
Trp Lys Ile Leu Asp Gln Ile Pro Gly Arg Ala Thr Gly Ala Tyr Ser
                165                 170                 175

cac agc cag ggt atc aag ggg tta cgt gat gca att gct gat gga atc      576
His Ser Gln Gly Ile Lys Gly Leu Arg Asp Ala Ile Ala Asp Gly Ile
            180                 185                 190

gaa gcc cgt gat ggt ttc cct gct gat cct aat gat ata ttc atg aca      624
Glu Ala Arg Asp Gly Phe Pro Ala Asp Pro Asn Asp Ile Phe Met Thr
        195                 200                 205

gat ggt gca agt cca ggg gta cat atg atg atg caa ctt ctc ata act      672
Asp Gly Ala Ser Pro Gly Val His Met Met Met Gln Leu Leu Ile Thr
    210                 215                 220

tca gag aaa gat gga atc ctt tgt cct att cct cag tat cca ttg tac      720
Ser Glu Lys Asp Gly Ile Leu Cys Pro Ile Pro Gln Tyr Pro Leu Tyr
225                 230                 235                 240

tca gct tca att gcc ctt cac ggt gga act ttg gtt cca tac tac ctt      768
Ser Ala Ser Ile Ala Leu His Gly Gly Thr Leu Val Pro Tyr Tyr Leu
                245                 250                 255

gat gaa gca tca gga tgg ggt ctt gaa ata tct gag ctg aag aaa caa      816
Asp Glu Ala Ser Gly Trp Gly Leu Glu Ile Ser Glu Leu Lys Lys Gln
            260                 265                 270

ctt gaa gat gct agg tca aag ggc atc act gtg aga gct ttg gct gtc      864
Leu Glu Asp Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Leu Ala Val
        275                 280                 285
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att aac cct gga aac ccg aca ggg cag gtt ctt tcg gaa gaa aac cag      912
Ile Asn Pro Gly Asn Pro Thr Gly Gln Val Leu Ser Glu Glu Asn Gln
    290                 295                 300

cgt gac gtt gtt aag ttc tgc aag caa gag ggt tta gtt ctt tta gca      960
Arg Asp Val Val Lys Phe Cys Lys Gln Glu Gly Leu Val Leu Leu Ala
305                 310                 315                 320

gac gag gtt tat caa gag aat gtc tat gtc cct gac aaa aag ttc cat     1008
Asp Glu Val Tyr Gln Glu Asn Val Tyr Val Pro Asp Lys Lys Phe His
                325                 330                 335

tcc ttc aag aaa gta gcc cgc tct atg ggc tac ggt gag aag gat ctt     1056
Ser Phe Lys Lys Val Ala Arg Ser Met Gly Tyr Gly Glu Lys Asp Leu
            340                 345                 350

gcc tta gtc tct ttc caa tct gtc tcc aaa ggg tac tat gga gag tgt     1104
Ala Leu Val Ser Phe Gln Ser Val Ser Lys Gly Tyr Tyr Gly Glu Cys
        355                 360                 365

ggg aaa aga ggt ggt tac atg gag gtt act gga ttc act tct gat gta     1152
Gly Lys Arg Gly Gly Tyr Met Glu Val Thr Gly Phe Thr Ser Asp Val
    370                 375                 380

aga gag cag ata tac aaa atg gct tct gtg aat ctt tgt tcc aac atc     1200
Arg Glu Gln Ile Tyr Lys Met Ala Ser Val Asn Leu Cys Ser Asn Ile
385                 390                 395                 400

tct ggt caa att ctt gct agc ctc atc atg agc cca ccc aag cct ggt     1248
Ser Gly Gln Ile Leu Ala Ser Leu Ile Met Ser Pro Pro Lys Pro Gly
                405                 410                 415

gac gac tcc tat gaa tca tac ata gca gag aag gat gga att ctc tca     1296
Asp Asp Ser Tyr Glu Ser Tyr Ile Ala Glu Lys Asp Gly Ile Leu Ser
            420                 425                 430

tct ttg gca aga cgt gca aag act ctt gaa gag gct ctg aac aag cta     1344
Ser Leu Ala Arg Arg Ala Lys Thr Leu Glu Glu Ala Leu Asn Lys Leu
        435                 440                 445

gag gga gtt aca tgc aat aga gca gaa gga gct atg tat cta ttc cct     1392
Glu Gly Val Thr Cys Asn Arg Ala Glu Gly Ala Met Tyr Leu Phe Pro
    450                 455                 460

tgc ctt cac ctt cca caa aag gca att gca gct gct gag gcg gaa aag     1440
Cys Leu His Leu Pro Gln Lys Ala Ile Ala Ala Ala Glu Ala Glu Lys
465                 470                 475                 480

aca gca cca gac aat ttc tac tgc aaa cgc ctt cta aaa gct act gga     1488
Thr Ala Pro Asp Asn Phe Tyr Cys Lys Arg Leu Leu Lys Ala Thr Gly
                485                 490                 495

ata gtc gtt gtc cct ggt tct ggc ttt aga cag gta cct gga aca tgg     1536
Ile Val Val Val Pro Gly Ser Gly Phe Arg Gln Val Pro Gly Thr Trp
            500                 505                 510
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cat ttc agg tgc act ata ctt ccc caa gag gat aag att cca gcg att 1584
His Phe Arg Cys Thr Ile Leu Pro Gln Glu Asp Lys Ile Pro Ala Ile
515 520 525

gtt gat cgt cta act gcg ttc cac cag agc ttc atg gac gag ttc cgc 1632
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Ile Pro Pro Phe Ser Ala Pro Ser Asp Ser Ser Ser Arg His Leu Ser
35 40 45

Ser Ser Ser Ser Ser Asp Met Ser Ala Ser Asp Ser Ser Ser Ser Leu
50 55 60

Pro Val Thr Leu Asp Thr Ile Asn Pro Lys Val Ile Lys Cys Glu Tyr
65 70 75 80

Ala Val Arg Gly Glu Ile Val Asn Ile Ala Gln Lys Leu Gln Glu Asp
85 90 95

Leu Lys Thr Asn Lys Asp Ala Tyr Pro Phe Asp Glu Ile Ile Tyr Cys
100 105 110

Asn Ile Gly Asn Pro Gln Ser Leu Gly Gln Gln Pro Ile Thr Phe Phe
115 120 125

Arg Glu Val Leu Ala Leu Cys Ser Tyr Thr Ala Leu Leu Asp Glu Ser
130 135 140

Ala Thr His Gly Leu Phe Arg Phe Ser Ser Asp Ser Ile Glu Arg Ala
145 150 155 160

Trp Lys Ile Leu Asp Gln Ile Pro Gly Arg Ala Thr Gly Ala Tyr Ser
165 170 175

His Ser Gln Gly Ile Lys Gly Leu Arg Asp Ala Ile Ala Asp Gly Ile
180 185 190

Glu Ala Arg Asp Gly Phe Pro Ala Asp Pro Asn Asp Ile Phe Met Thr
195 200 205

Asp Gly Ala Ser Pro Gly Val His Met Met Met Gln Leu Leu Ile Thr
210 215 220

Ser Glu Lys Asp Gly Ile Leu Cys Pro Ile Pro Gln Tyr Pro Leu Tyr
225 230 235 240

Ser Ala Ser Ile Ala Leu His Gly Gly Thr Leu Val Pro Tyr Tyr Leu
245 250 255

Asp Glu Ala Ser Gly Trp Gly Leu Glu Ile Ser Glu Leu Lys Lys Gln
260 265 270

Leu Glu Asp Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Leu Ala Val
275 280 285

Ile Asn Pro Gly Asn Pro Thr Gly Gln Val Leu Ser Glu Glu Asn Gln
290 295 300

Arg Asp Val Val Lys Phe Cys Lys Gln Glu Gly Leu Val Leu Leu Ala
305 310 315 320

Asp Glu Val Tyr Gln Glu Asn Val Tyr Val Pro Asp Lys Lys Phe His
325 330 335

Ser Phe Lys Lys Val Ala Arg Ser Met Gly Tyr Gly Glu Lys Asp Leu
340 345 350

Ala Leu Val Ser Phe Gln Ser Val Ser Lys Gly Tyr Tyr Gly Glu Cys
355 360 365

Gly Lys Arg Gly Gly Tyr Met Glu Val Thr Gly Phe Thr Ser Asp Val
370 375 380

Arg Glu Gln Ile Tyr Lys Met Ala Ser Val Asn Leu Cys Ser Asn Ile
385 390 395 400

Ser Gly Gln Ile Leu Ala Ser Leu Ile Met Ser Pro Pro Lys Pro Gly
405 410 415

Asp Asp Ser Tyr Glu Ser Tyr Ile Ala Glu Lys Asp Gly Ile Leu Ser
420 425 430

Ser Leu Ala Arg Arg Ala Lys Thr Leu Glu Glu Ala Leu Asn Lys Leu
435 440 445

Glu Gly Val Thr Cys Asn Arg Ala Glu Gly Ala Met Tyr Leu Phe Pro
450 455 460

Cys Leu His Leu Pro Gln Lys Ala Ile Ala Ala Ala Glu Ala Glu Lys
465 470 475 480

Thr Ala Pro Asp Asn Phe Tyr Cys Lys Arg Leu Leu Lys Ala Thr Gly
485 490 495

Ile Val Val Val Pro Gly Ser Gly Phe Arg Gln Val Pro Gly Thr Trp
500 505 510

His Phe Arg Cys Thr Ile Leu Pro Gln Glu Asp Lys Ile Pro Ala Ile
515 520 525

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Asp
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<400> 30
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gag ctg aac gag aac gtg aag aag gtg cag tac gcg gtg cgg ggg gag 96
Glu Leu Asn Glu Asn Val Lys Lys Val Gln Tyr Ala Val Arg Gly Glu
20 25 30

ctg tac ctg cgc gcc tcc gag ctc cag aag gag ggc aag aag atc atc 144
Leu Tyr Leu Arg Ala Ser Glu Leu Gln Lys Glu Gly Lys Lys Ile Ile
35 40 45

ttc acc aac gtc ggc aac cca cac gcg ctc ggc cag aag ccg ctc acc 192
Phe Thr Asn Val Gly Asn Pro His Ala Leu Gly Gln Lys Pro Leu Thr
50 55 60

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ttc ccc cgc cag gtt gtg gcg ctg tgc cag gcc ccc ttc ctg ctc gat      240
Phe Pro Arg Gln Val Val Ala Leu Cys Gln Ala Pro Phe Leu Leu Asp
65                  70                  75                  80

gat ccc aac gtc ggc ctt atc ttc ccc gcc gac gcc atc gcg cgg gcc      288
Asp Pro Asn Val Gly Leu Ile Phe Pro Ala Asp Ala Ile Ala Arg Ala
                85                  90                  95

aag cac tac ctc gcc atg gca ccc ggt gga cta gct tac agt gat tcc      336
Lys His Tyr Leu Ala Met Ala Pro Gly Gly Leu Ala Tyr Ser Asp Ser
            100                 105                 110

cga ggt atc cct ggt att agg aag gaa gtc gcc gag ttc atc gag agg      384
Arg Gly Ile Pro Gly Ile Arg Lys Glu Val Ala Glu Phe Ile Glu Arg
        115                 120                 125

cgt gat ggt tat cca gat cca gaa ctt att tac ctc aca gat ggt gcc      432
Arg Asp Gly Tyr Pro Asp Pro Glu Leu Ile Tyr Leu Thr Asp Gly Ala
    130                 135                 140

agc aaa ggt gtg atg caa atg ctg aat acc att atc aga aat gag aga      480
Ser Lys Gly Val Met Gln Met Leu Asn Thr Ile Ile Arg Asn Glu Arg
145                 150                 155                 160

gat ggg att ctg gtt cct gtt cca caa tac ccg ctt tat tct gct gcc      528
Asp Gly Ile Leu Val Pro Val Pro Gln Tyr Pro Leu Tyr Ser Ala Ala
                165                 170                 175

att tcc ctc ttt ggt ggt tct ctc gtg cca tac tac tta gaa gaa gag      576
Ile Ser Leu Phe Gly Gly Ser Leu Val Pro Tyr Tyr Leu Glu Glu Glu
            180                 185                 190

gct aac tgg gga ctt gac ttc gtc aat ctc cga cag act gtg gcg tca      624
Ala Asn Trp Gly Leu Asp Phe Val Asn Leu Arg Gln Thr Val Ala Ser
        195                 200                 205

gcg cgg tca aag gga atc act gtt cga gca atg gtg att atc aac cca      672
Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Met Val Ile Ile Asn Pro
    210                 215                 220

gga aac cct act ggc caa tgc ctt agt gaa gga aac ata aag gaa ctt      720
Gly Asn Pro Thr Gly Gln Cys Leu Ser Glu Gly Asn Ile Lys Glu Leu
225                 230                 235                 240

ctc aaa ttc tgc ttc cat gag aac tta gtt ctg ctt gca gat gaa gtc      768
Leu Lys Phe Cys Phe His Glu Asn Leu Val Leu Leu Ala Asp Glu Val
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tat caa cag aac att tat caa gat gag cgc cca ttt ata agt gct aga      816
Tyr Gln Gln Asn Ile Tyr Gln Asp Glu Arg Pro Phe Ile Ser Ala Arg
            260                 265                 270

aag gtt ctg ttt gac atg ggt cct cct atg agc agg gaa gtt cag ctg      864
Lys Val Leu Phe Asp Met Gly Pro Pro Met Ser Arg Glu Val Gln Leu
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36aa

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Arg Gly Gly Tyr Phe Glu Met Thr Asn Leu Pro Pro Lys Thr Val Asp
305                 310                 315                 320

gag atc tac aag gtt gca tca atc gca ctc agt cca aat gtt cct ggg     1008
Glu Ile Tyr Lys Val Ala Ser Ile Ala Leu Ser Pro Asn Val Pro Gly
                325                 330                 335

cag atc ttt atg ggt tta atg gtt aac cct cct aag cct gga gat atc     1056
Gln Ile Phe Met Gly Leu Met Val Asn Pro Pro Lys Pro Gly Asp Ile
            340                 345                 350

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Ser Tyr Leu Lys Phe Ser Ala Glu Lys Ser Ile Leu Glu Ser Leu Arg
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agg aga gca cgc ctg atg aca gat ggt ttc aat agt tgc cga aat gtt     1152
Arg Arg Ala Arg Leu Met Thr Asp Gly Phe Asn Ser Cys Arg Asn Val
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aag ttc aac gac act ttc atg gat cag tac gat ggc tac tcc agg atg     1440
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35 40 45

Phe Thr Asn Val Gly Asn Pro His Ala Leu Gly Gln Lys Pro Leu Thr
50 55 60

Phe Pro Arg Gln Val Val Ala Leu Cys Gln Ala Pro Phe Leu Leu Asp
65 70 75 80

Asp Pro Asn Val Gly Leu Ile Phe Pro Ala Asp Ala Ile Ala Arg Ala
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Lys His Tyr Leu Ala Met Ala Pro Gly Gly Leu Ala Tyr Ser Asp Ser
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Arg Gly Ile Pro Gly Ile Arg Lys Glu Val Ala Glu Phe Ile Glu Arg
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Arg Asp Gly Tyr Pro Asp Pro Glu Leu Ile Tyr Leu Thr Asp Gly Ala
130 135 140

Ser Lys Gly Val Met Gln Met Leu Asn Thr Ile Ile Arg Asn Glu Arg
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Asp Gly Ile Leu Val Pro Val Pro Gln Tyr Pro Leu Tyr Ser Ala Ala
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Ile Ser Leu Phe Gly Gly Ser Leu Val Pro Tyr Tyr Leu Glu Glu Glu
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Ala Asn Trp Gly Leu Asp Phe Val Asn Leu Arg Gln Thr Val Ala Ser
195 200 205

Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Met Val Ile Ile Asn Pro
210 215 220

Gly Asn Pro Thr Gly Gln Cys Leu Ser Glu Gly Asn Ile Lys Glu Leu
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Leu Lys Phe Cys Phe His Glu Asn Leu Val Leu Leu Ala Asp Glu Val
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Tyr Gln Gln Asn Ile Tyr Gln Asp Glu Arg Pro Phe Ile Ser Ala Arg
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Lys Val Leu Phe Asp Met Gly Pro Pro Met Ser Arg Glu Val Gln Leu
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Arg Gly Gly Tyr Phe Glu Met Thr Asn Leu Pro Pro Lys Thr Val Asp
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Glu Ile Tyr Lys Val Ala Ser Ile Ala Leu Ser Pro Asn Val Pro Gly
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340 345 350

Ser Tyr Leu Lys Phe Ser Ala Glu Lys Ser Ile Leu Glu Ser Leu Arg
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370 375 380

Val Cys Asn Phe Thr Glu Ala Met Tyr Ser Phe Pro Gln Ile Arg Leu
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Pro Pro Lys Ala Ile Asp Ala Ala Lys Arg Ala Gly Lys Ala Ala Asp
405 410 415

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Pro Gly Ser Gly Phe Gly Gln Lys Glu Val Phe His Leu Arg Thr Thr
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65                  70                  75                  80

ccc aac gtc ggc ctt atc ttc ccc gcc gac gcc atc gcg cgg gcc aag      288
Pro Asn Val Gly Leu Ile Phe Pro Ala Asp Ala Ile Ala Arg Ala Lys
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cac tac ctc gcc atg gca ccc ggt gga cta ggt gct tac agt gat tcc      336
His Tyr Leu Ala Met Ala Pro Gly Gly Leu Gly Ala Tyr Ser Asp Ser
            100                 105                 110

cga ggt atc cct ggt att agg aag gaa gtc gcc gag ttc atc gag agg      384
Arg Gly Ile Pro Gly Ile Arg Lys Glu Val Ala Glu Phe Ile Glu Arg
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Arg Asp Gly Tyr Pro Ser Asp Pro Glu Leu Ile Tyr Leu Thr Asp Gly
    130                 135                 140

gcc agc aaa ggt gtg atg caa atg ctg aat acc att atc aga aat gag      480
Ala Ser Lys Gly Val Met Gln Met Leu Asn Thr Ile Ile Arg Asn Glu
145                 150                 155                 160

aga gat ggg att ctg gtt cct gtt cca caa tac ccg ctt tat tct gct      528
Arg Asp Gly Ile Leu Val Pro Val Pro Gln Tyr Pro Leu Tyr Ser Ala
                165                 170                 175

gcc att tcc ccc ttt ggt ggt tct ctc gtg cca tac tac tta gaa gaa      576
Ala Ile Ser Pro Phe Gly Gly Ser Leu Val Pro Tyr Tyr Leu Glu Glu
            180                 185                 190

gag gct aac tgg gga ctt gac ttc gtc aat ctc cga cag act gtg gcg      624
Glu Ala Asn Trp Gly Leu Asp Phe Val Asn Leu Arg Gln Thr Val Ala
        195                 200                 205

tca gcg cgg tca aag gga atc act gtt cga gca atg gtg att atc aac      672
Ser Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Met Val Ile Ile Asn
    210                 215                 220

cca gga aac cct act ggc caa tgc ctt agt gaa gga aac ata aag gaa      720
Pro Gly Asn Pro Thr Gly Gln Cys Leu Ser Glu Gly Asn Ile Lys Glu
225                 230                 235                 240

ctt ctc aaa ttc tgc ttc cat gag aac tta gtt ctg ctt gca gat gaa      768
Leu Leu Lys Phe Cys Phe His Glu Asn Leu Val Leu Leu Ala Asp Glu
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Val Tyr Gln Gln Asn Ile Tyr Gln Asp Glu Arg Pro Phe Ile Ser Ala
            260                 265                 270

aga aag gtt ctg ttt gac atg ggt cct cct atg agc agg gaa gtt cag      864
Arg Lys Val Leu Phe Asp Met Gly Pro Pro Met Ser Arg Glu Val Gln
        275                 280                 285

ctg gtt tct ttc cat act gtg tca aaa gga tat tgg ggg gag tgt gga      912
Leu Val Ser Phe His Thr Val Ser Lys Gly Tyr Trp Gly Glu Cys Gly
    290                 295                 300

caa cgt gga ggg tat ttt gaa atg aca aat ctt cct ccc aag aca gta      960
Gln Arg Gly Gly Tyr Phe Glu Met Thr Asn Leu Pro Pro Lys Thr Val
305                 310                 315                 320

gac gag atc tac aag gtt gca tca atc gca ctc agt cca aat gtt cct     1008
Asp Glu Ile Tyr Lys Val Ala Ser Ile Ala Leu Ser Pro Asn Val Pro
                325                 330                 335

ggg cag atc ttt atg ggt tta atg gtt aac cct cct aag cct gga gat     1056
Gly Gln Ile Phe Met Gly Leu Met Val Asn Pro Pro Lys Pro Gly Asp
            340                 345                 350

atc tct tat ctg aag ttt tct gct gaa agc aag tct atc ctc gag tct     1104
Ile Ser Tyr Leu Lys Phe Ser Ala Glu Ser Lys Ser Ile Leu Glu Ser
        355                 360                 365

ttg agg agg aga gca cgc ctg atg aca gat ggt ttc aat agt tgc cga     1152
Leu Arg Arg Arg Ala Arg Leu Met Thr Asp Gly Phe Asn Ser Cys Arg
    370                 375                 380

aat gtt gtc tgc aat ttc aca gaa gga gct atg tac tct ttc ccc caa     1200
Asn Val Val Cys Asn Phe Thr Glu Gly Ala Met Tyr Ser Phe Pro Gln
385                 390                 395                 400

ata cgc tta cca cca aaa gct ata gat gca gcc aaa agg gct ggc aaa     1248
Ile Arg Leu Pro Pro Lys Ala Ile Asp Ala Ala Lys Arg Ala Gly Lys
                405                 410                 415

gcg gcc gat gtt ttc tac tgc ctc aag ctt ctt gaa gca act gga ata     1296
Ala Ala Asp Val Phe Tyr Cys Leu Lys Leu Leu Glu Ala Thr Gly Ile
            420                 425                 430

tcc act gtt cca ggg tca ggt ttc gga caa aaa gaa ggg gtg ttc cac     1344
Ser Thr Val Pro Gly Ser Gly Phe Gly Gln Lys Glu Gly Val Phe His
        435                 440                 445

ctg agg acg acc atc ctg cca gct gag gag gac atg cct gcc atc atg     1392
Leu Arg Thr Thr Ile Leu Pro Ala Glu Glu Asp Met Pro Ala Ile Met
    450                 455                 460

acc agc ttc aag aag ttc aac gac act ttc atg gat cag tac gat ggc     1440
Thr Ser Phe Lys Lys Phe Asn Asp Thr Phe Met Asp Gln Tyr Asp Gly
465                 470                 475                 480
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Thr Asn Val Gly Asn Pro His Ala Leu Gly Gln Lys Pro Leu Thr Phe
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Pro Arg Gln Val Val Ala Leu Cys Gln Ala Pro Phe Leu Leu Asp Asp
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Pro Asn Val Gly Leu Ile Phe Pro Ala Asp Ala Ile Ala Arg Ala Lys
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His Tyr Leu Ala Met Ala Pro Gly Gly Leu Gly Ala Tyr Ser Asp Ser
100 105 110

Arg Gly Ile Pro Gly Ile Arg Lys Glu Val Ala Glu Phe Ile Glu Arg
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Arg Asp Gly Tyr Pro Ser Asp Pro Glu Leu Ile Tyr Leu Thr Asp Gly
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Ala Ser Lys Gly Val Met Gln Met Leu Asn Thr Ile Ile Arg Asn Glu
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Ala Ile Ser Pro Phe Gly Gly Ser Leu Val Pro Tyr Tyr Leu Glu Glu
180 185 190

Glu Ala Asn Trp Gly Leu Asp Phe Val Asn Leu Arg Gln Thr Val Ala
195 200 205

Ser Ala Arg Ser Lys Gly Ile Thr Val Arg Ala Met Val Ile Ile Asn
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Pro Gly Asn Pro Thr Gly Gln Cys Leu Ser Glu Gly Asn Ile Lys Glu
225 230 235 240

Leu Leu Lys Phe Cys Phe His Glu Asn Leu Val Leu Leu Ala Asp Glu
245 250 255

Val Tyr Gln Gln Asn Ile Tyr Gln Asp Glu Arg Pro Phe Ile Ser Ala
260 265 270

Arg Lys Val Leu Phe Asp Met Gly Pro Pro Met Ser Arg Glu Val Gln
275 280 285

Leu Val Ser Phe His Thr Val Ser Lys Gly Tyr Trp Gly Glu Cys Gly
290 295 300

Gln Arg Gly Gly Tyr Phe Glu Met Thr Asn Leu Pro Pro Lys Thr Val
305 310 315 320

Asp Glu Ile Tyr Lys Val Ala Ser Ile Ala Leu Ser Pro Asn Val Pro
325 330 335

Gly Gln Ile Phe Met Gly Leu Met Val Asn Pro Pro Lys Pro Gly Asp
340 345 350

Ile Ser Tyr Leu Lys Phe Ser Ala Glu Ser Lys Ser Ile Leu Glu Ser
355 360 365

Leu Arg Arg Arg Ala Arg Leu Met Thr Asp Gly Phe Asn Ser Cys Arg
370 375 380

Asn Val Val Cys Asn Phe Thr Glu Gly Ala Met Tyr Ser Phe Pro Gln
385 390 395 400

Ile Arg Leu Pro Pro Lys Ala Ile Asp Ala Ala Lys Arg Ala Gly Lys
405 410 415

Ala Ala Asp Val Phe Tyr Cys Leu Lys Leu Leu Glu Ala Thr Gly Ile
420 425 430

Ser Thr Val Pro Gly Ser Gly Phe Gly Gln Lys Glu Gly Val Phe His
435 440 445

Leu Arg Thr Thr Ile Leu Pro Ala Glu Glu Asp Met Pro Ala Ile Met
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Tyr Ser Arg Met

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What is claimed:

1. A method of increasing an amino acid content in a plant or in a seed of the plant, said method comprising the step of introducing a genetic construct for the expression of a glutamate glyoxylate aminotransferase (GGT) gene into a cell of the plant to produce a transgenic plant, wherein:

- the GGT gene encodes a polypeptide having GGT activity in a peroxisome;
- the C-terminal of the polypeptide encoded by the GGT gene has an amino acid sequence of [Ser or Ala]-[Arg or Lys]-[Ile or Leu or Met];
- the complement sequence of the GGT gene has a nucleotide sequence which hybridizes, under a stringent condition, to the polynucleotide of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent condition comprises a washing step at 50°C in 2X SSC and 0.1% SDS;
- the genetic construct increases the GGT activity of the transgenic plant as compared with a corresponding non-transformed plant which is cultivated under the same condition;
- the content of at least one amino acids selected from the group consisting of serine, arginine, glutamine and asparagine in the transgenic plant is increased as compared with the corresponding non-transformed plant which is cultivated under the same condition.

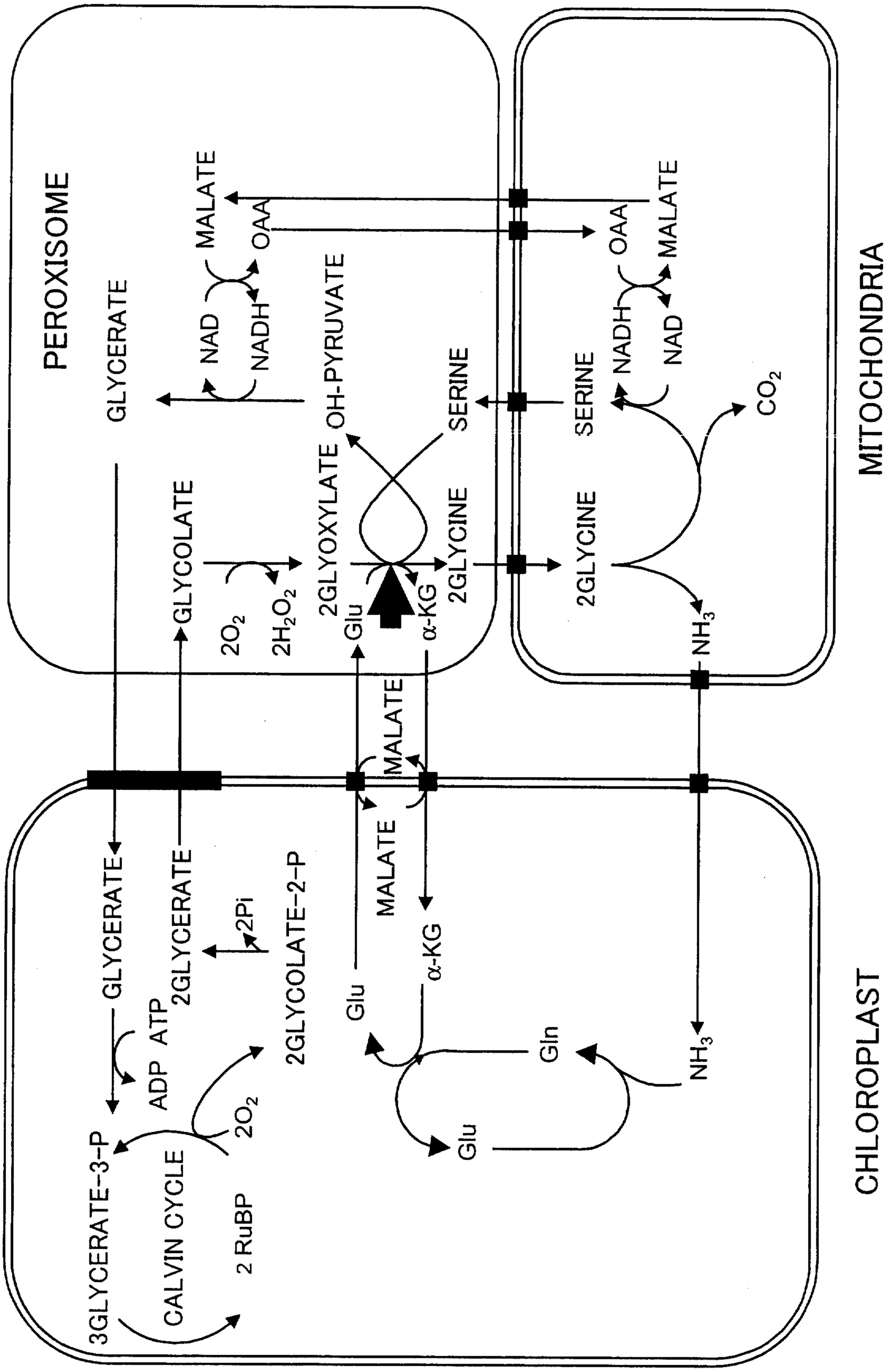
2. The method according to claim 1, wherein the GGT gene has the nucleotide sequence of SEQ ID NO:1.

3. The method according to claim 1, wherein the GGT gene has the nucleotide sequence of SEQ ID NO:3.

4. The method according to claim 1, wherein the polypeptide encoded by the GGT gene has the amino acid sequence of SEQ ID NO:2.

5. The method according to claim 1, wherein the polypeptide encoded by the GGT gene has the amino acid sequence of SEQ ID NO:4.

FIG. 1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ADP ATP
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MALATE
NH_3
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Glu
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CALVIN CYCLE
$2O_2$
2 RuBP
CHLOROPLAST

FIG. 2

```
GGT1    1:------------------------------------------------------------ 0
GGT2    1:------------------------------------------------------------ 0
GGT3    1:MRRFLINQAKGLVDHS-RRQ-HHHKSPSFLSPQPRPLASSPPALSRFF--SSTSEMSASD 56
GGT4    1:MRRFVIGQAKNLIDQSRRRQLHHHKNLSFVSLIP-PFSAPSDSSSRHLSSSSSSDMSASD 59

GGT1    1:-MALKALDYDTLNENVKKCQYAVRGE-LYL--R-ASEL--QKEG---KKVIFTNVGNPHA 50
GGT2    1:-MSLKALDYESLNENVKNCQYAVRGE-LYL--R-ASEL--QKEG---KKIIFTNVGNPHA 50
GGT3   57:STSSLPVTLDSINPKVLKCEYAVRGEIVNIAQKLQEDLKTNKDAYPFDEIIYCNIGNPQS 116
GGT4   60:SSSSLPVTLDTINPKVIKCEYAVRGEIVNIAQKLQEDLKTNKDAYPFDEIIYCNIGNPQS 119
                  *  *  * ******           *   *        *  * ***

GGT1   51:LGQKPLTFPRQVVALCQAPFLLDDPNV-GM-LFPADAIARAKHYLSLTSG-GLGAYSDSR 107
GGT2   51:LGQKPLTFPRQVVSLCQAPFLLDDPNV-GM-IFPADAIARAKHYLSLTSG-GLGAYSDSR 107
GGT3  117:LGQLPIKFFREVLALCDHASLLDESETHGL--FSTDSIDRAWRILDHIPGRATGAYSHSQ 174
GGT4  120:LGQQPITFFREVLALCSYTALLDESATHGLFRFSSDSIERAWKILDQIPGRATGAYSHSQ 179
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GGT1  108:GLPGVRKEVAEFIQRRDGYPSDPELIFLTDGASKGVMQILNCVIRGNGDGILVPVPQYPL 167
GGT2  108:GLPGVRKEVAEFIERRDGYPSDPELIFLTDGASKGVMQILNCVIRGQKDGILVPVPQYPL 167
GGT3  175:GIKGLRDVIAAGIEARDGFPADPNDIFLTDGASPAVHMMMQLLLSSEKDGILSPIPQYPL 234
GGT4  180:GIKGLRDAIADGIEARDGFPADPNDIFMTDGASPGVHMMMQLLITSEKDGILCPIPQYPL 239
          *  * *   *  *  *** * **  ** *****  *                **** * *****

GGT1  168:YSATISLLGGTLVPYYLDESENWGLDVANLRQSVAQARSQGITVRAMVIINPGNPTGQCL 227
GGT2  168:YSATISLLGGTLVPYYLEESENWGLDVNNLRQSVAQARSQGITVRAMVIINPGNPTGQCL 227
GGT3  235:YSASIALHGGSLVPYYLDEATGWGLEISDLKKQLEEARSKGISVRALVVINPGNPTGQVL 294
GGT4  240:YSASIALHGGTLVPYYLDEASGWGLEISELKKQLEDARSKGITVRALAVINPGNPTGQVL 299
          *** * * ** ****** *   ***    *       *** ** ***   ********* *

GGT1  228:SEANIREILKFCYNEKLVLLGDEVYQQNIYQDERPFISSKKVLMEMGSPFSKEVQLVSFH 287
GGT2  228:SEANIREILRFCCDERLVLLGDEVYQQNIYQDERPFISSKKVLMDMGAPISKEVQLISFH 287
GGT3  295:AEENQRDIVNFCKQEGLVLLADEVYQENVYVPDKKFHSFKKVARSLGY-GEKDISLVSFQ 353
GGT4  300:SEENQRDVVKFCKQEGLVLLADEVYQENVYVPDKKFHSFKKVARSMGY-GEKDLALVSFQ 358
            * * *    **  * **** ***** * *     * * ***    *    *   * **

GGT1  288:TVSKGYWGECGQRGGYFEMTNLPPRVVEEIYKVASIALSPNVSAQIFMGLMVNPPKPGDI 347
GGT2  288:TVSKGYWGECGQRGGYFEMTNIPPRTVEEIYKVASIALSPNVSAQIFMGLMVSPPKPGDI 347
GGT3  354:SVSKGYYGECGKRGGYMEVTGFTSDVREQIYKMASVNLCSNISGQILASLVMSPPKPGDD 413
GGT4  359:SVSKGYYGECGKRGGYMEVTGFTSDVREQIYKMASVNLCSNISGQILASLIMSPPKPGDD 418
          ***** **** **** * *       * *** **  *  * * **   *   ******

GGT1  348:SYDQFARESKGILESLRRRARLMTDGFNSCKNVVCNFTEGAMYSFPQIRLPTGALQAAKQ 407
GGT2  348:SYDQFVRESKGILESLRRRARMMTDGFNSCKNVVCNFTEGAMYSFPQIKLPSKAIQAAKQ 407
GGT3  414:SYDSYMAERDGILSSMAKRAKTLEDALNSLEGVTCNRAEGAMYLFPRINLPQKAIEAAEA 473
GGT4  419:SYESYIAEKDGILSSLARRAKTLEEALNKLEGVTCNRAEGAMYLFPCLHLPQKAIAAAEA 478
          **     *  *** *   **        *    * **  ***** **   **  *  **

GGT1  408:AGKVPDVFYCLKLLEATGISTVPGSGFGQKEGVFHLRTTILPAEDEMPEIMDSFKKFNDE 467
GGT2  408:AGKVPDVFYCLKLLEATGISTVPGSGFGQKEGVFHLRTTILPAEEEMPEIMDSFKKFNDE 467
GGT3  474:EKTAPDAFYCKRLLNATGVVVVPGSGFGQVPGTWHFRCTILPQEDKIPAIVNRLTEFHKS 533
GGT4  479:EKTAPDNFYCKRLLKATGIVVVPGSGFRQVPGTWHFRCTILPQEDKIPAIVDRLTAFHQS 538
            ** ***  ** ***   ****** *  *  * * **** *   * *      *

GGT1  468:FMTQYDNNFGYSKM                                              481
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GGT3  534:FMDEFRN-------                                              540
GGT4  539:FMDEFRD-------                                              545
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```
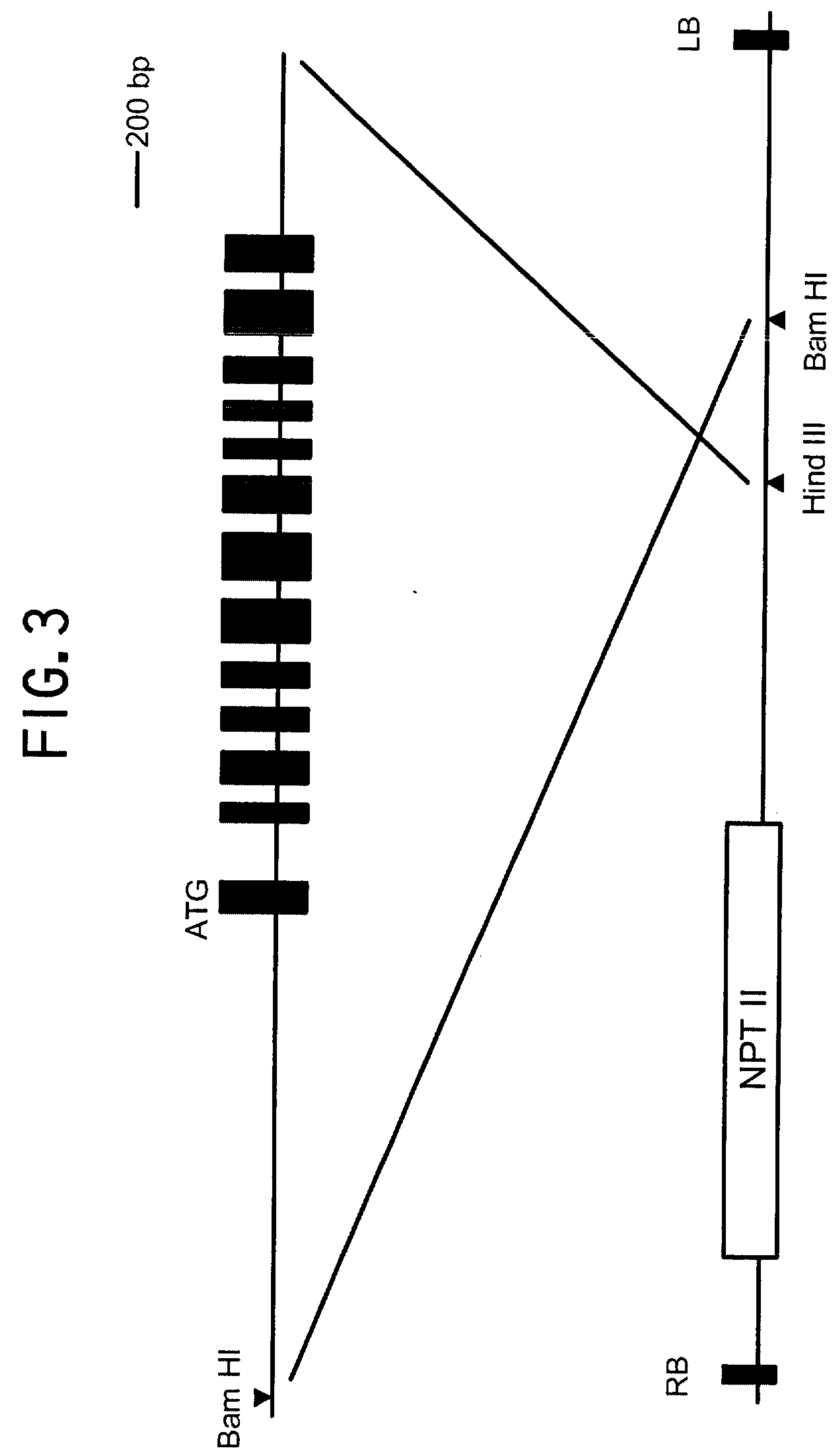



FIG. 3

FIG. 4

	WEIGHT OF 95 SEEDLINGS
ggt1-1/GGT1	628 mg
CONTROL	530 mg

FIG. 5

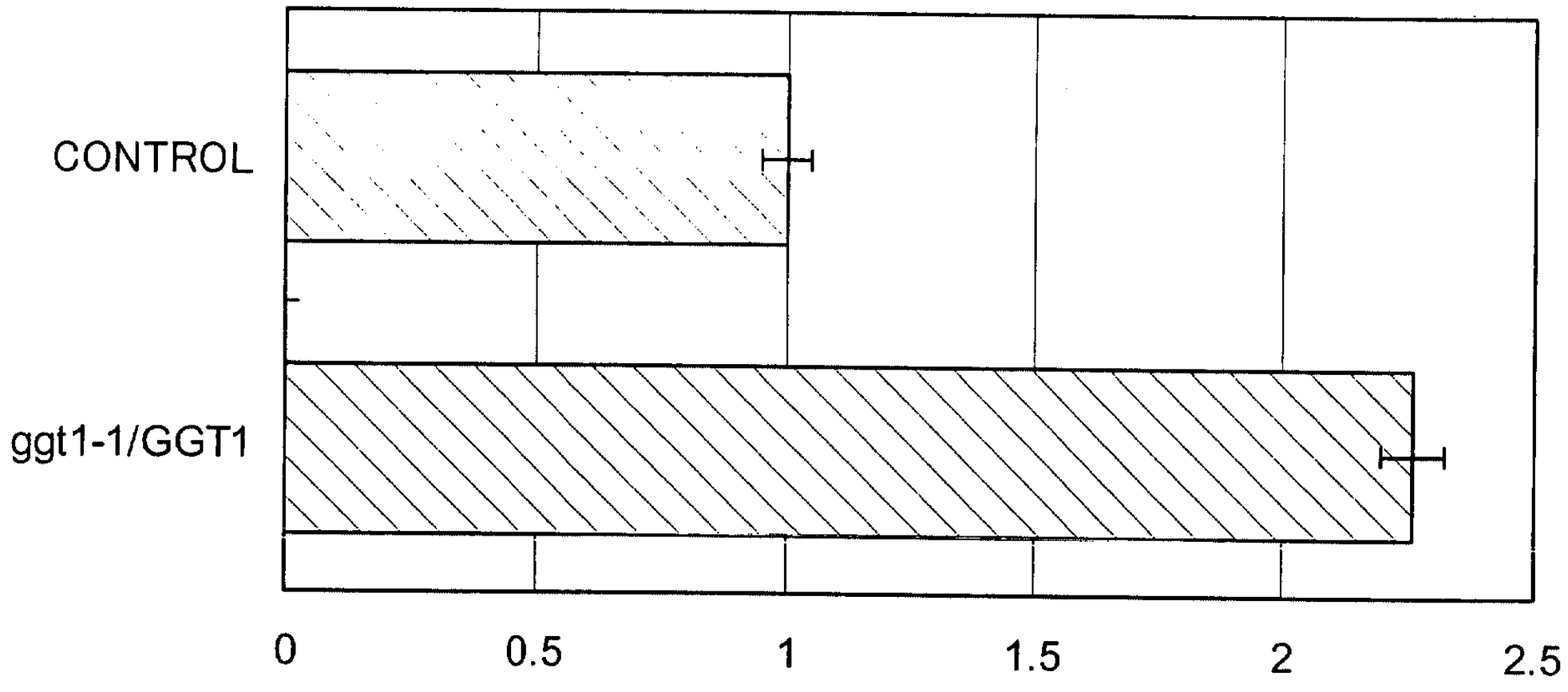

CONTROL
ggt1-1/GGT1
0
0.5
1
1.5
2
2.5

FIG. 6

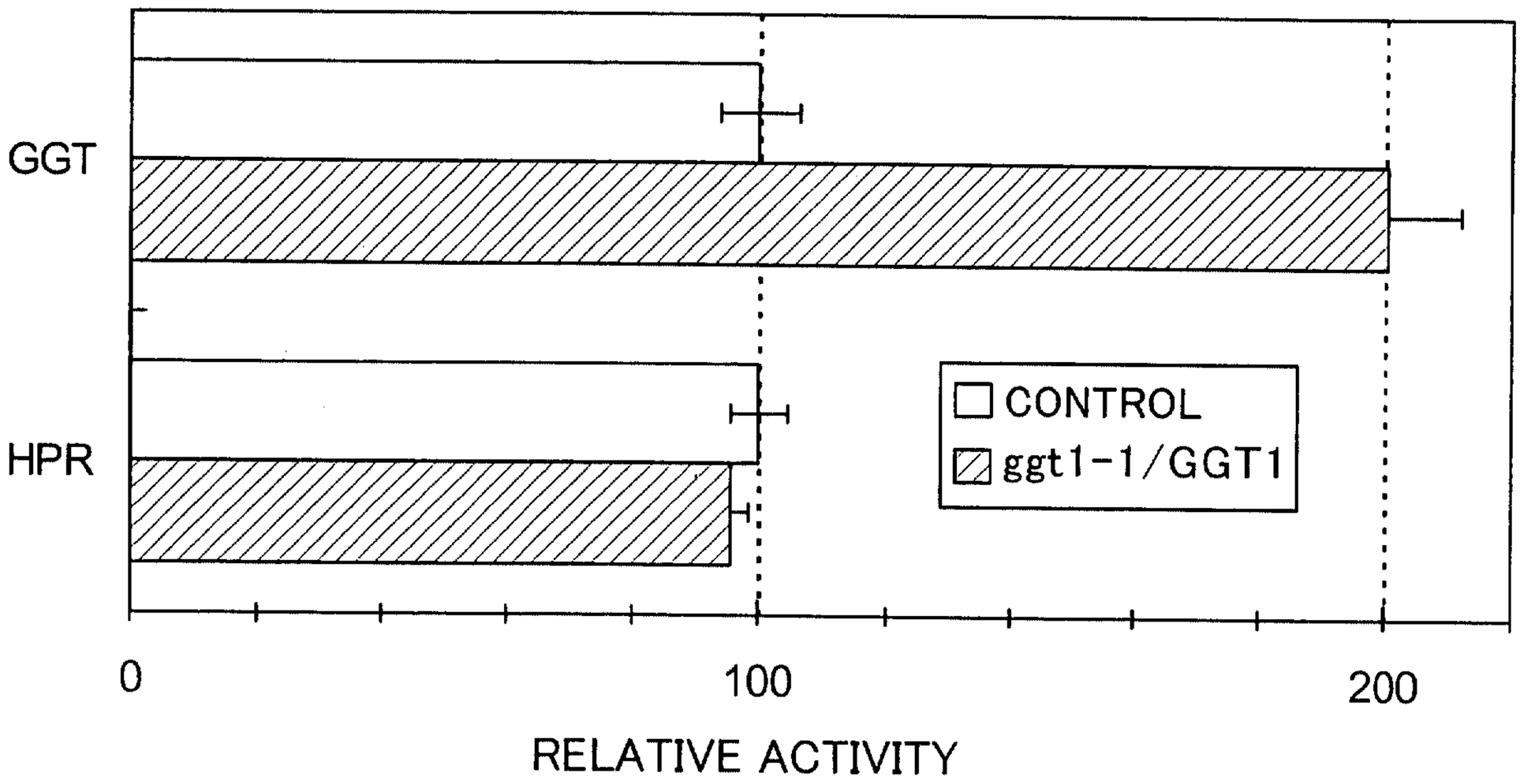
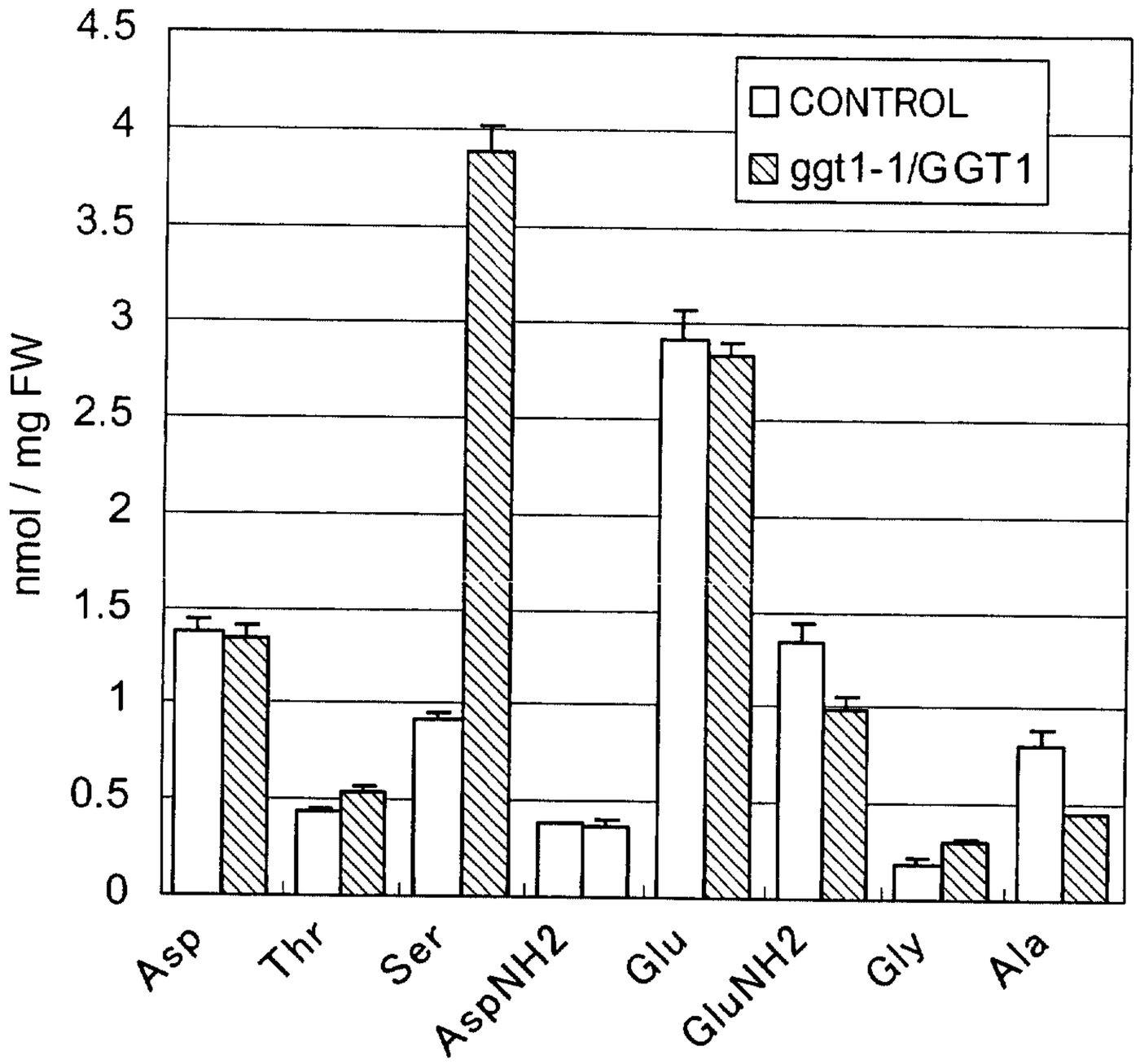

GGT
HPR
CONTROL
ggt1-1/GGT1
0
100
200
RELATIVE ACTIVITY

FIG. 7

(A)


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
nmol / mg FW
CONTROL
ggt1-1/GGT1
Asp
Thr
Ser
AspNH2
Glu
GluNH2
Gly
Ala

(B)

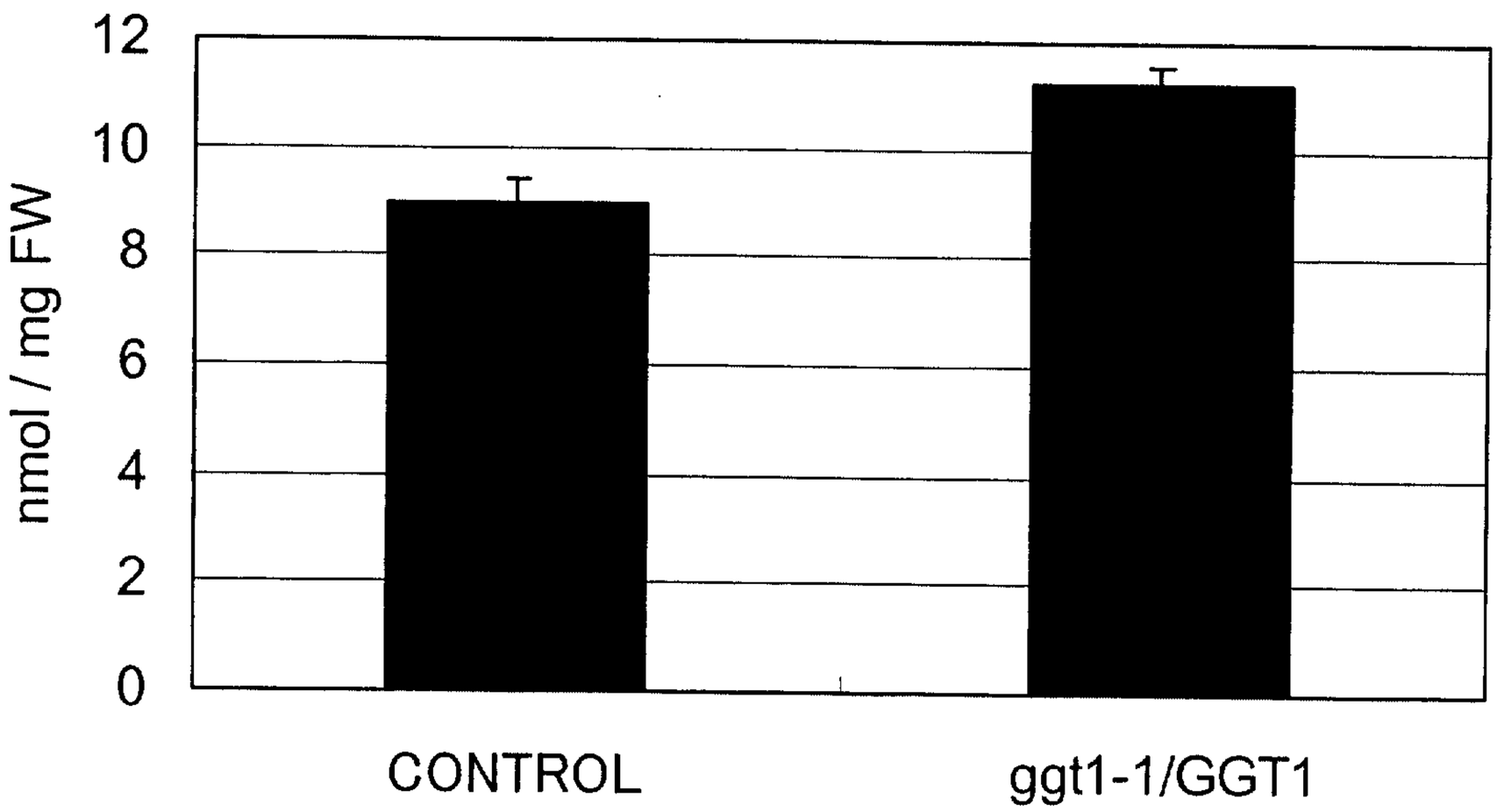
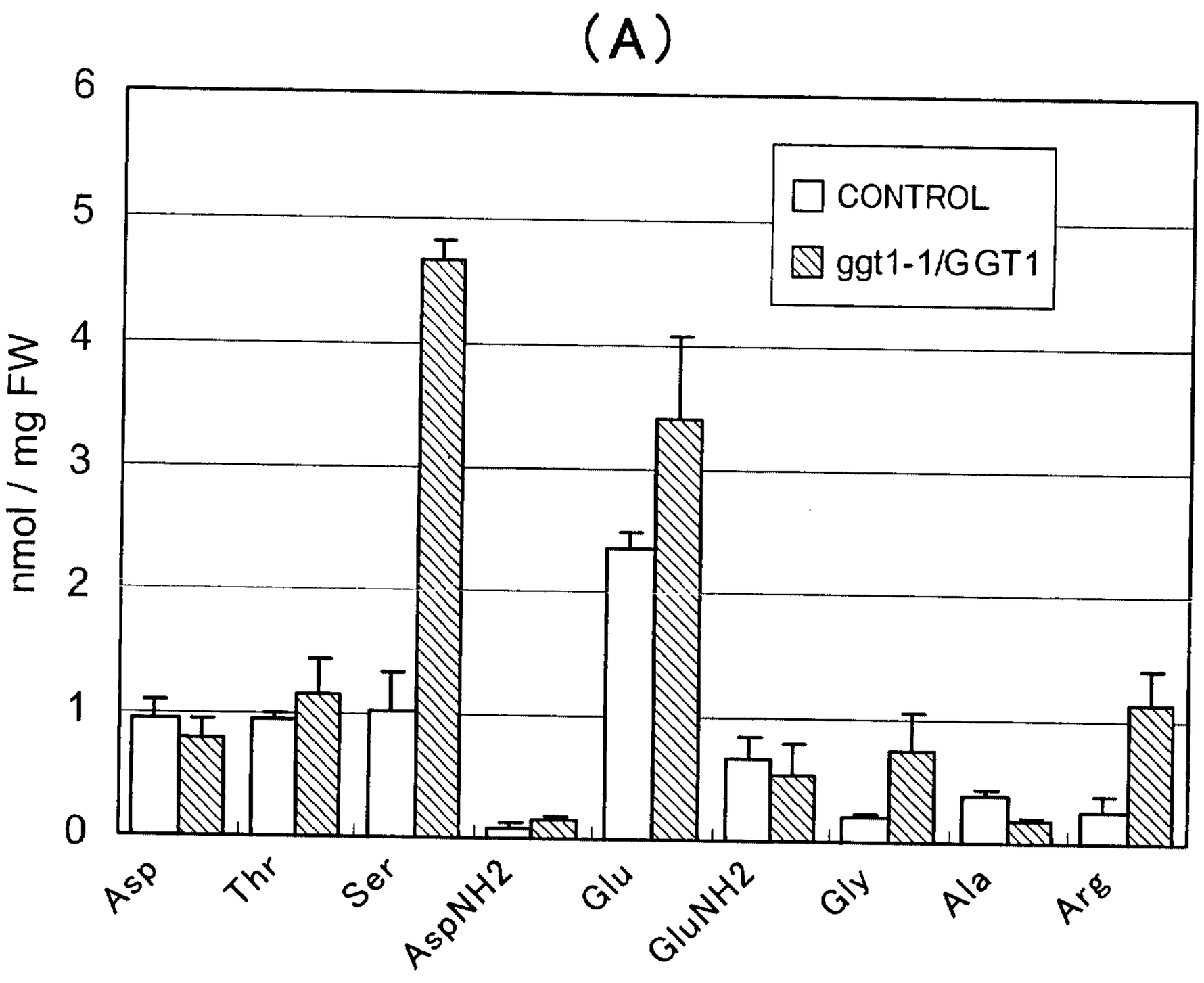

12
10
8
6
4
2
0
nmol / mg FW
CONTROL
ggt1-1/GGT1

FIG. 8


(A)
6
5
4
3
2
1
0
nmol / mg FW
CONTROL
ggt1-1/GGT1
Asp
Thr
Ser
AspNH2
Glu
GluNH2
Gly
Ala
Arg

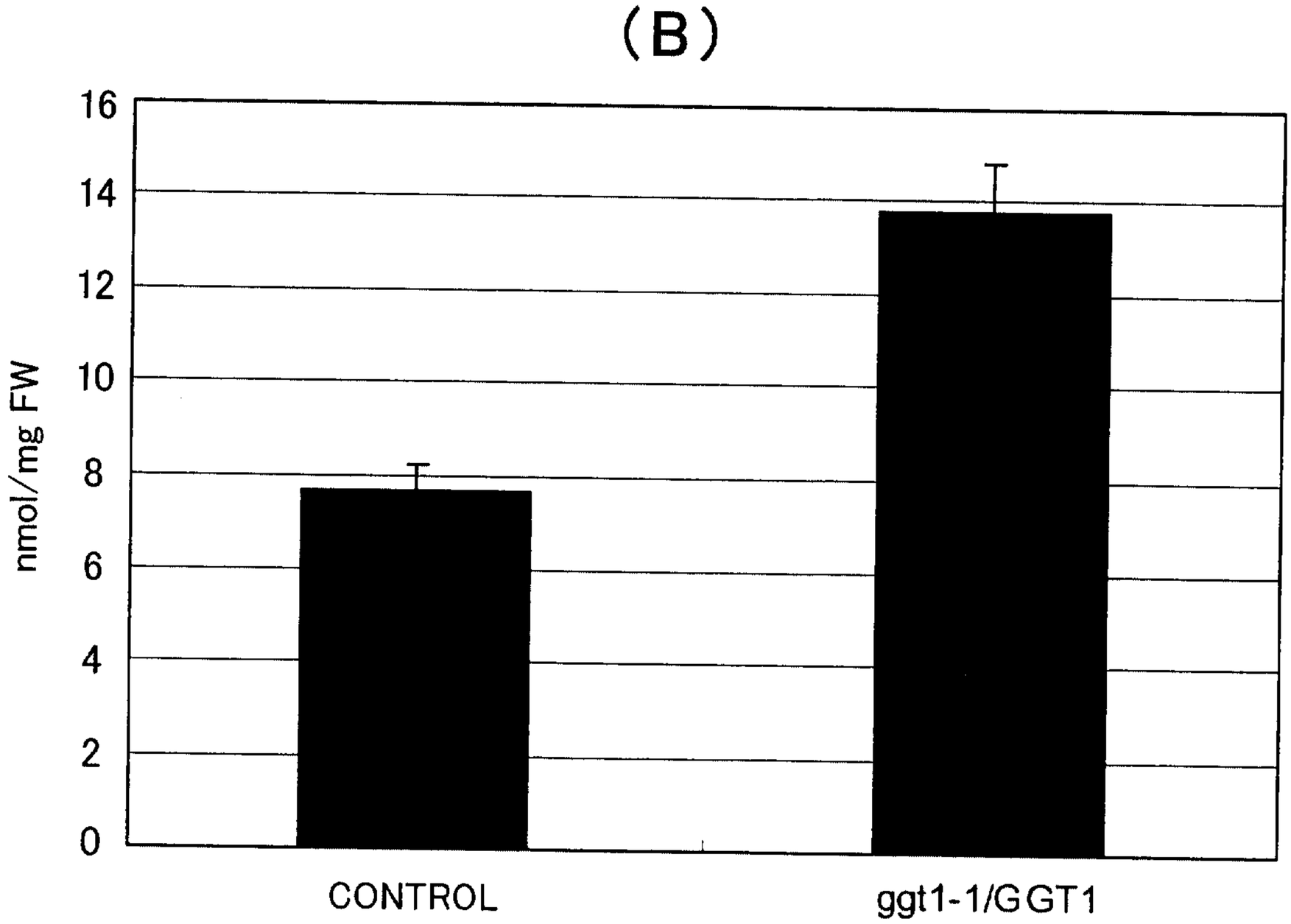

(B)
16
14
12
10
8
6
4
2
0
nmol/mg FW
CONTROL
ggt1-1/GGT1

FIG. 9

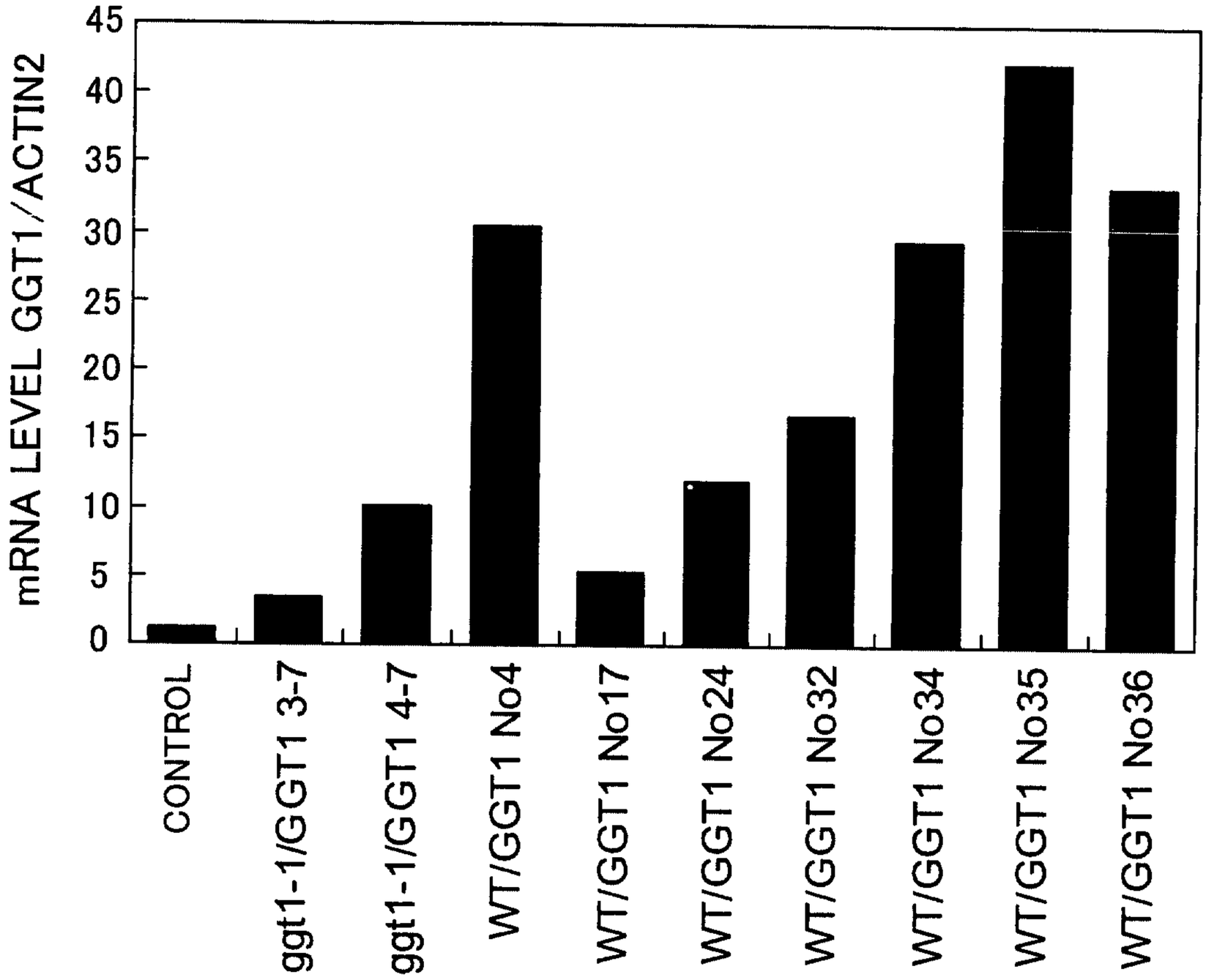
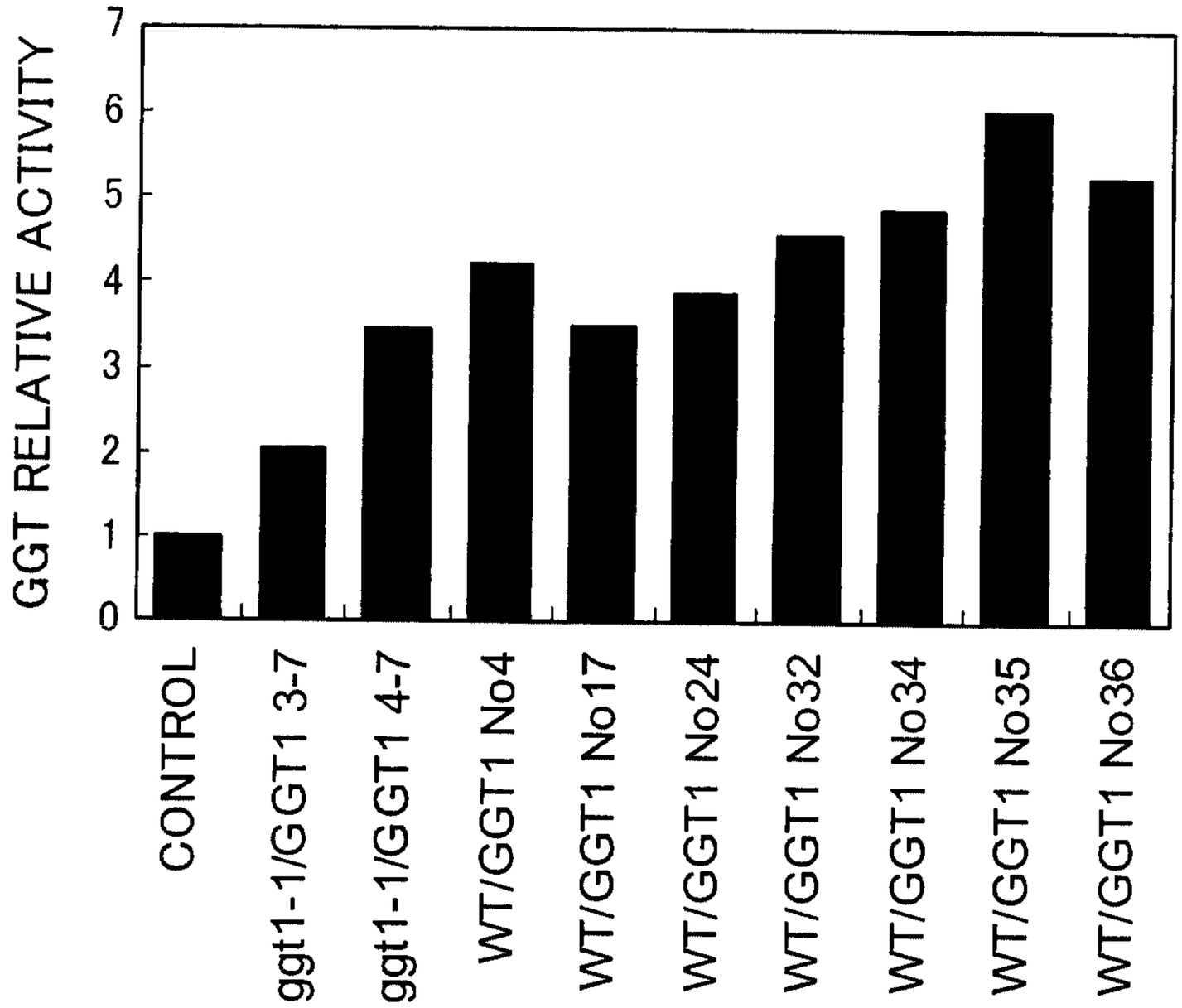

mRNA LEVEL GGT1/ACTIN2
0
5
10
15
20
25
30
35
40
45
CONTROL
ggt1-1/GGT1 3-7
ggt1-1/GGT1 4-7
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24
WT/GGT1 No32
WT/GGT1 No34
WT/GGT1 No35
WT/GGT1 No36

FIG. 10

(A)


GGT RELATIVE ACTIVITY
0
1
2
3
4
5
6
7
CONTROL
ggt1-1/GGT1 3-7
ggt1-1/GGT1 4-7
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24
WT/GGT1 No32
WT/GGT1 No34
WT/GGT1 No35
WT/GGT1 No36

(B)

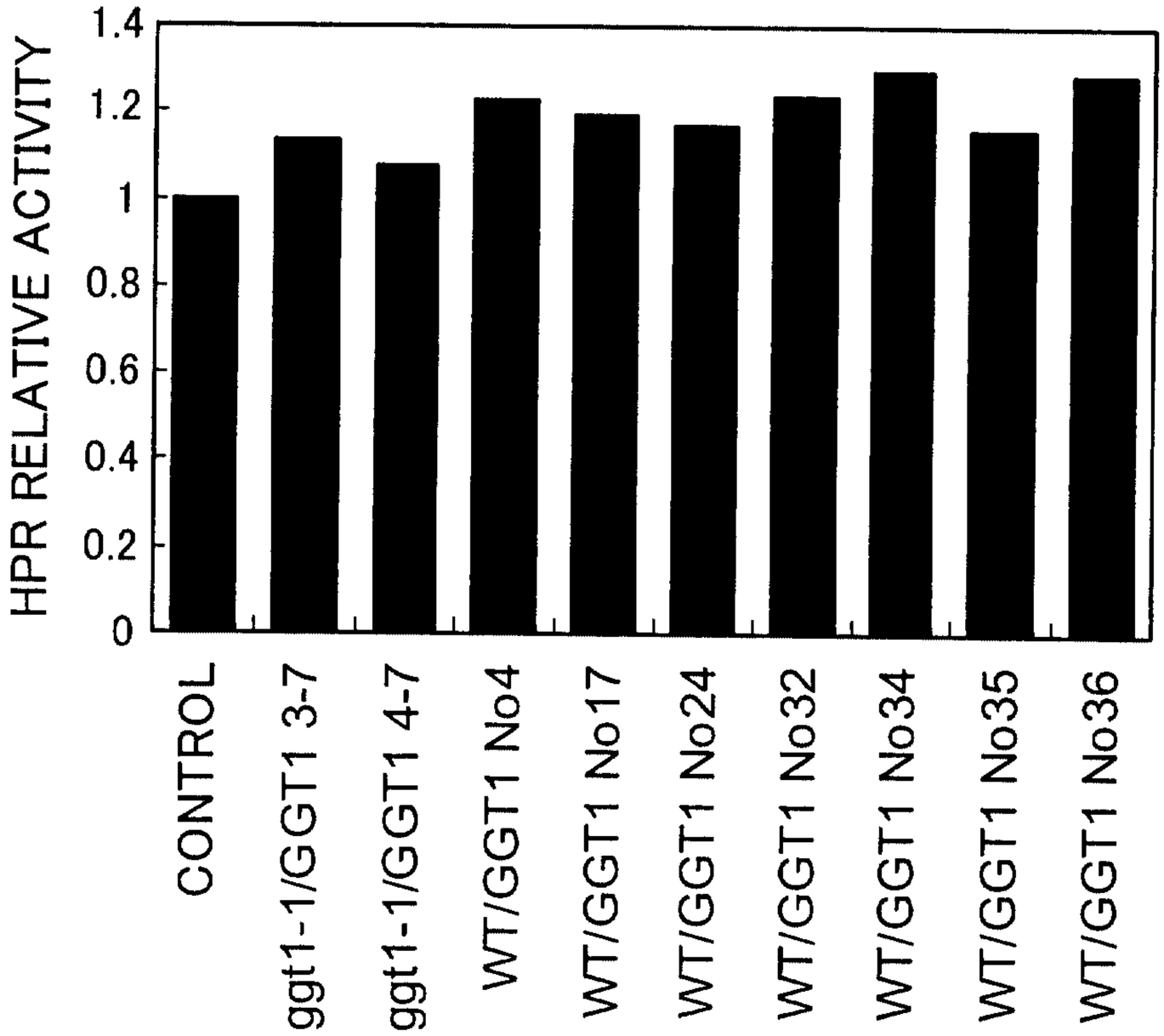

HPR RELATIVE ACTIVITY
0
0.2
0.4
0.6
0.8
1
1.2
1.4
CONTROL
ggt1-1/GGT1 3-7
ggt1-1/GGT1 4-7
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24
WT/GGT1 No32
WT/GGT1 No34
WT/GGT1 No35
WT/GGT1 No36

FIG. 11

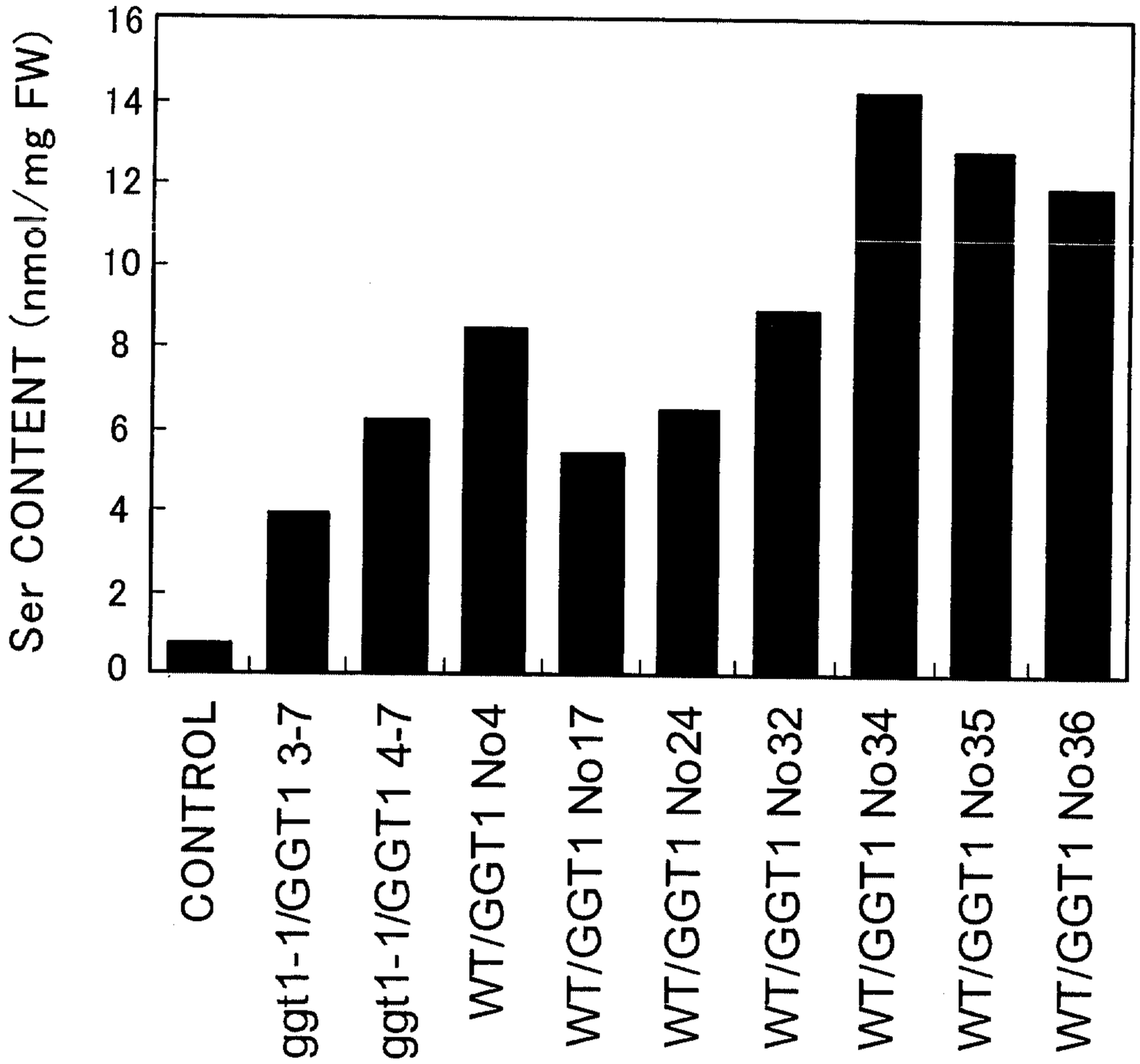
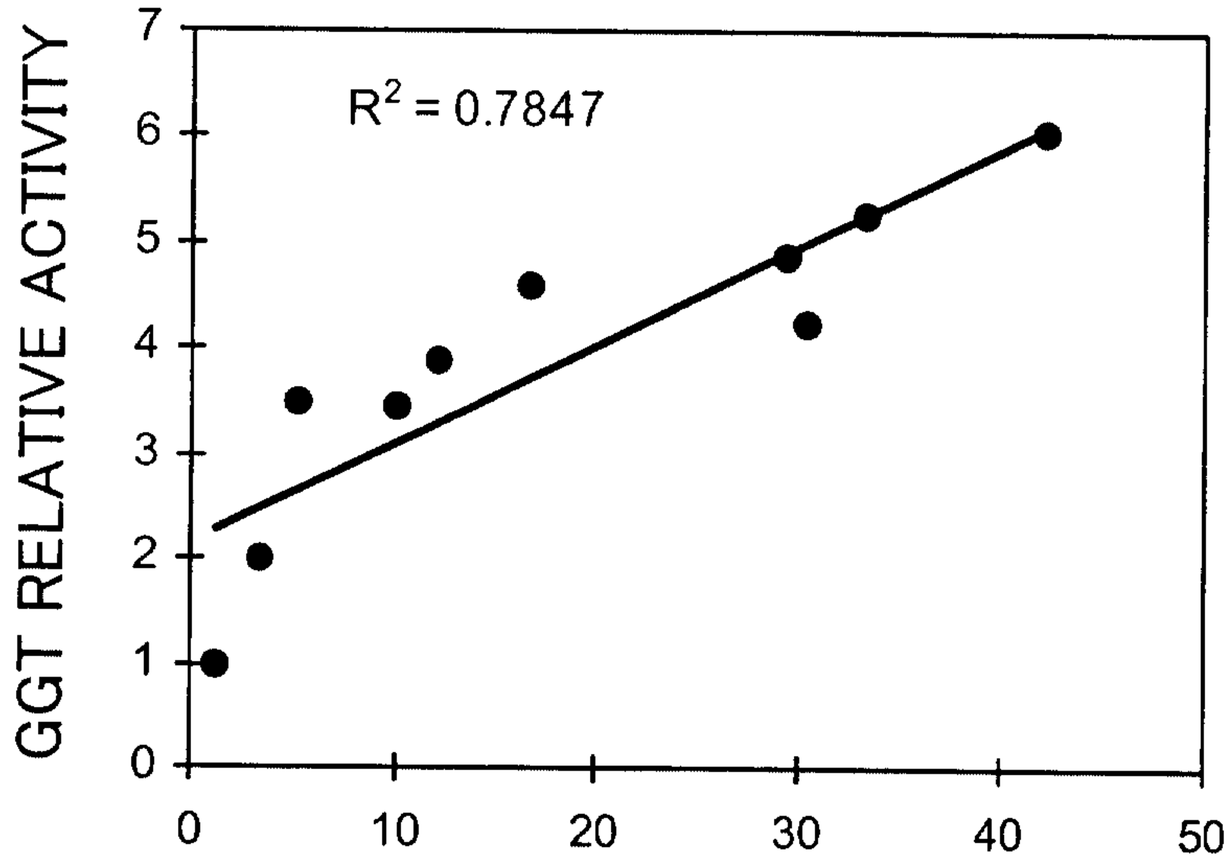
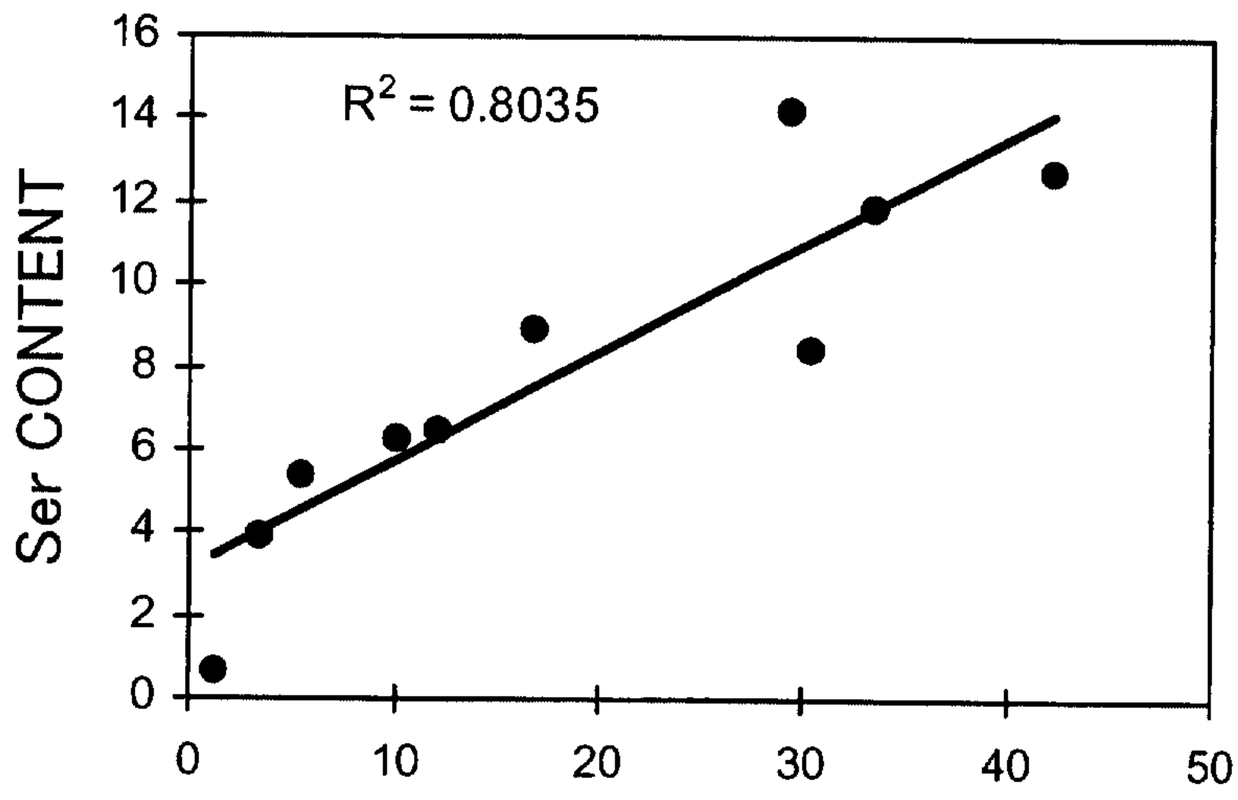

Ser CONTENT (nmol/mg FW)
0
2
4
6
8
10
12
14
16
CONTROL
ggt1-1/GGT1 3-7
ggt1-1/GGT1 4-7
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24
WT/GGT1 No32
WT/GGT1 No34
WT/GGT1 No35
WT/GGT1 No36

FIG. 12

(A)


R^2 = 0.7847
GGT RELATIVE ACTIVITY
0
1
2
3
4
5
6
7
0
10
20
30
40
50
GGT1 mRNA RELATIVE AMOUNT

(B)


R^2 = 0.8035
Ser CONTENT
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
GGT1 mRNA RELATIVE AMOUNT

(C)

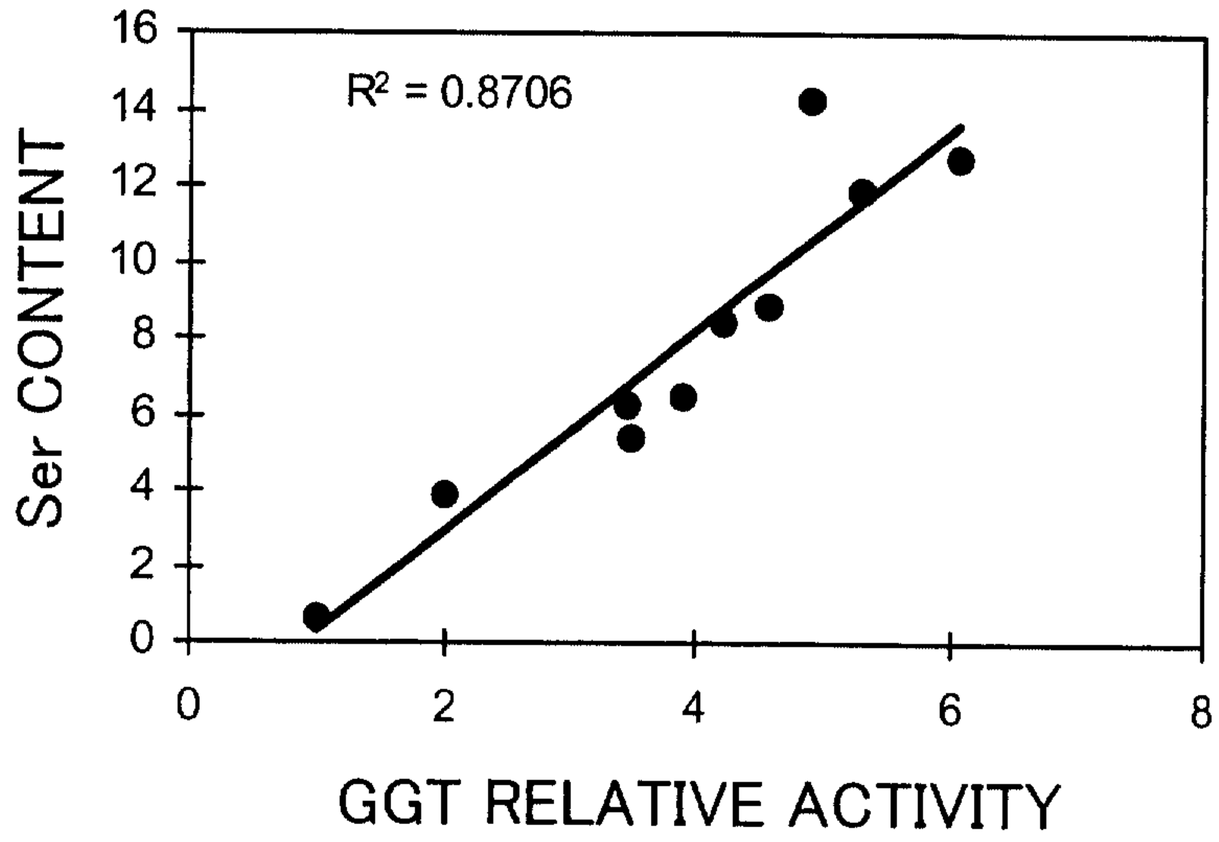
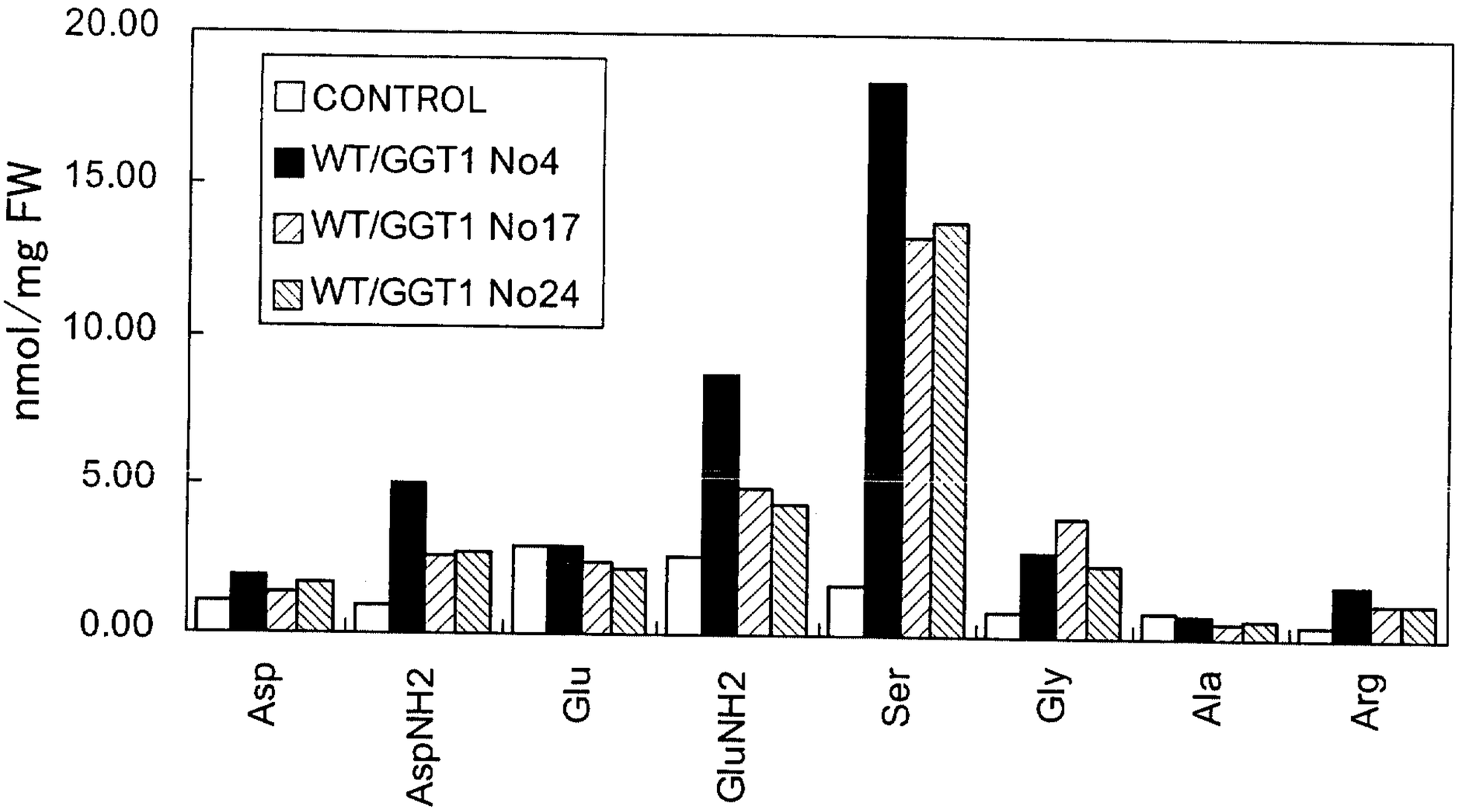

R^2 = 0.8706
Ser CONTENT
0
2
4
6
8
10
12
14
16
0
2
4
6
8
GGT RELATIVE ACTIVITY

FIG. 13

(A)


20.00
15.00
10.00
5.00
0.00
nmol/mg FW
CONTROL
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24
Asp
AspNH2
Glu
GluNH2
Ser
Gly
Ala
Arg

(B)

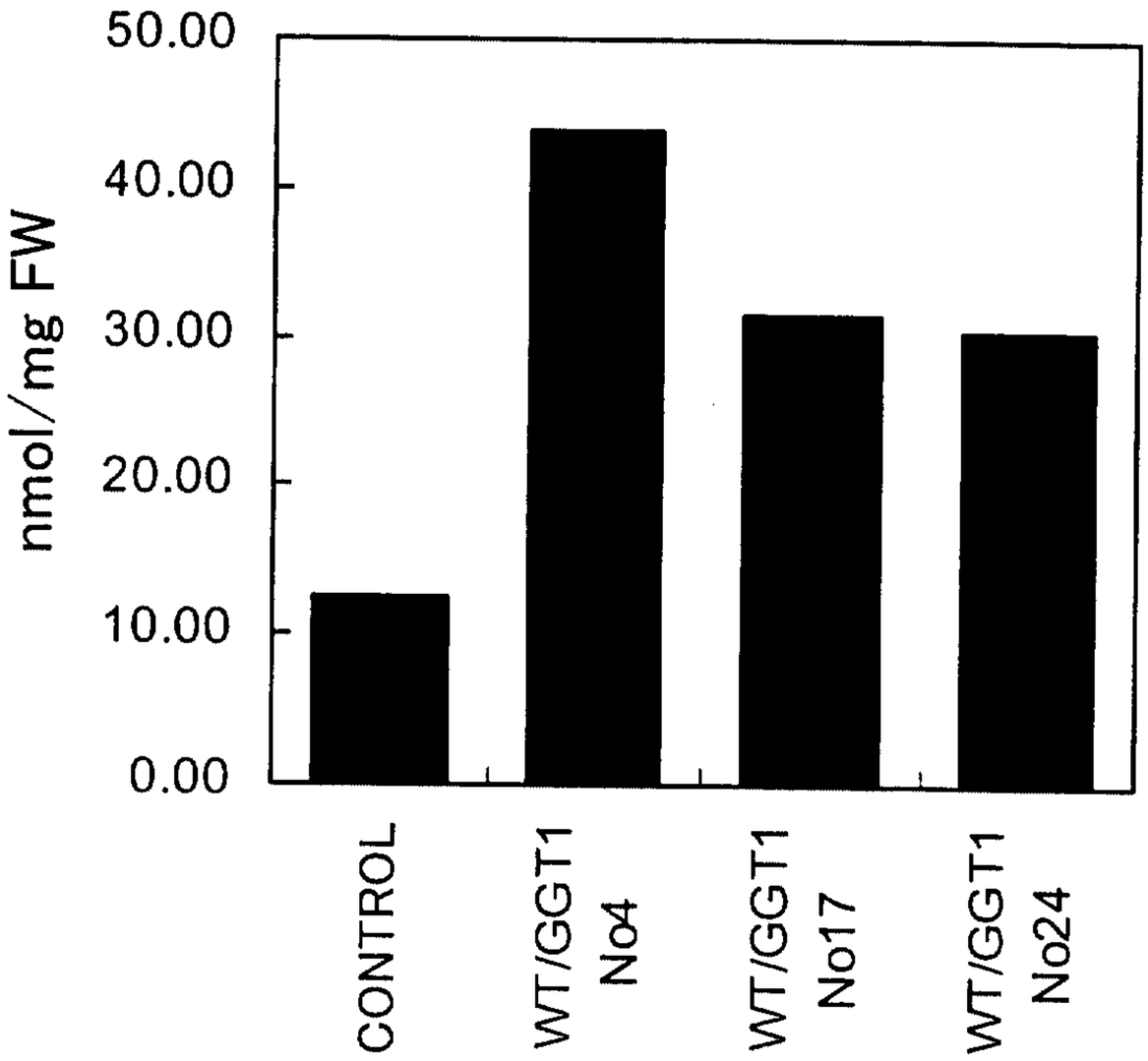
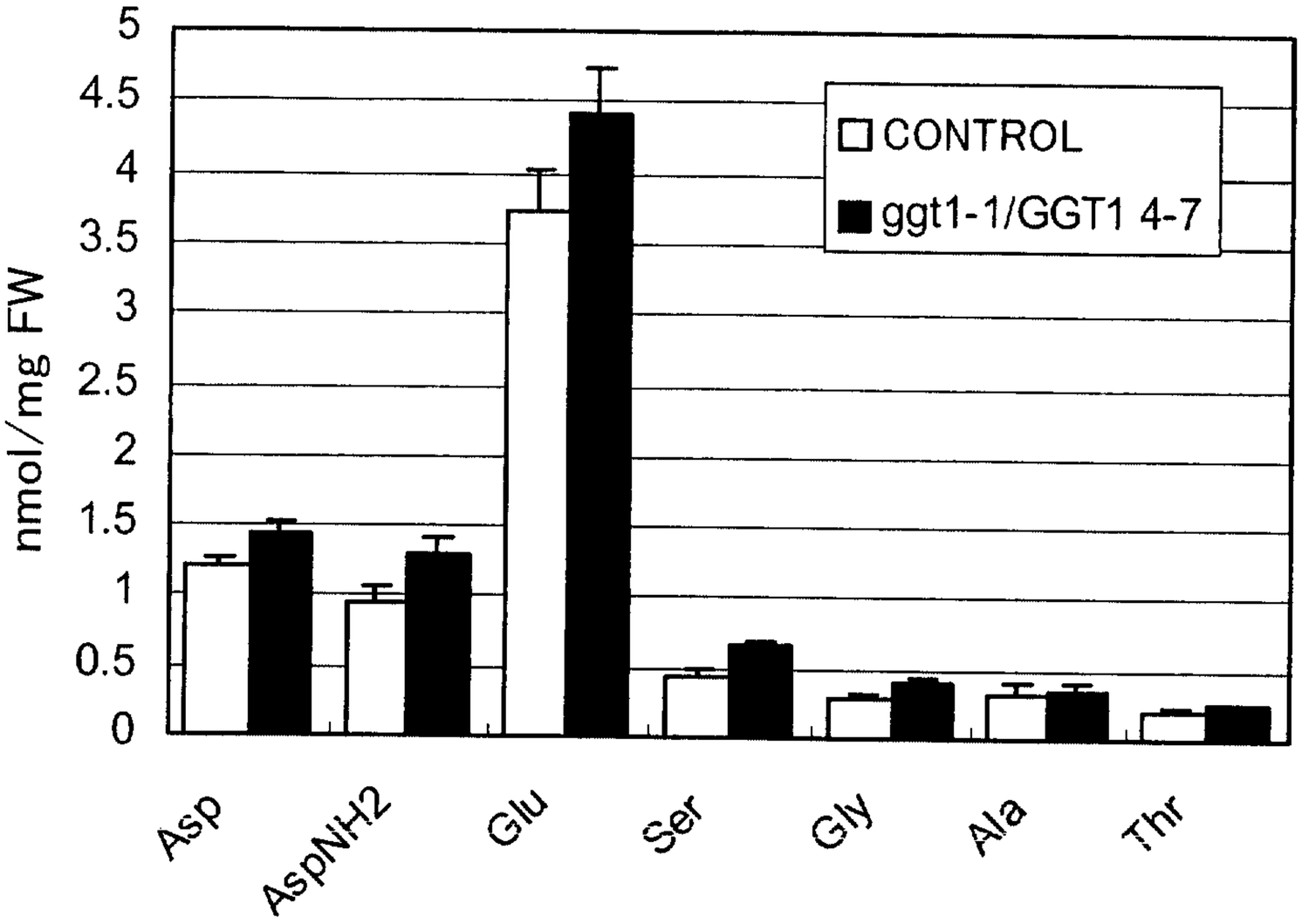
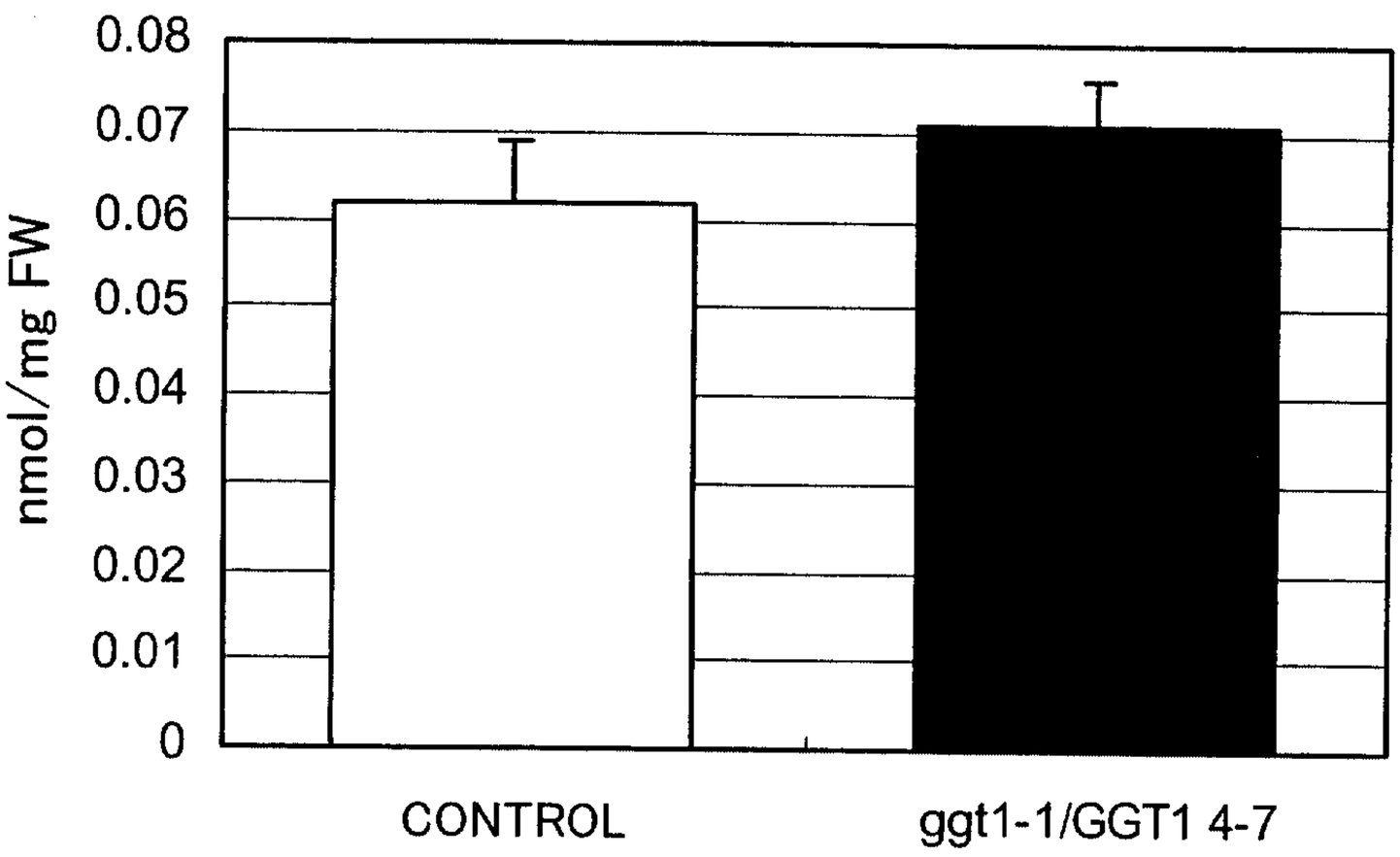

50.00
40.00
30.00
20.00
10.00
0.00
nmol/mg FW
CONTROL
WT/GGT1 No4
WT/GGT1 No17
WT/GGT1 No24

FIG. 14

(A)


nmol/mg FW
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
ggt1-1/GGT1 4-7
Asp
AspNH2
Glu
Ser
Gly
Ala
Thr

(B)


nmol/mg FW
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
CONTROL
ggt1-1/GGT1 4-7

(C)

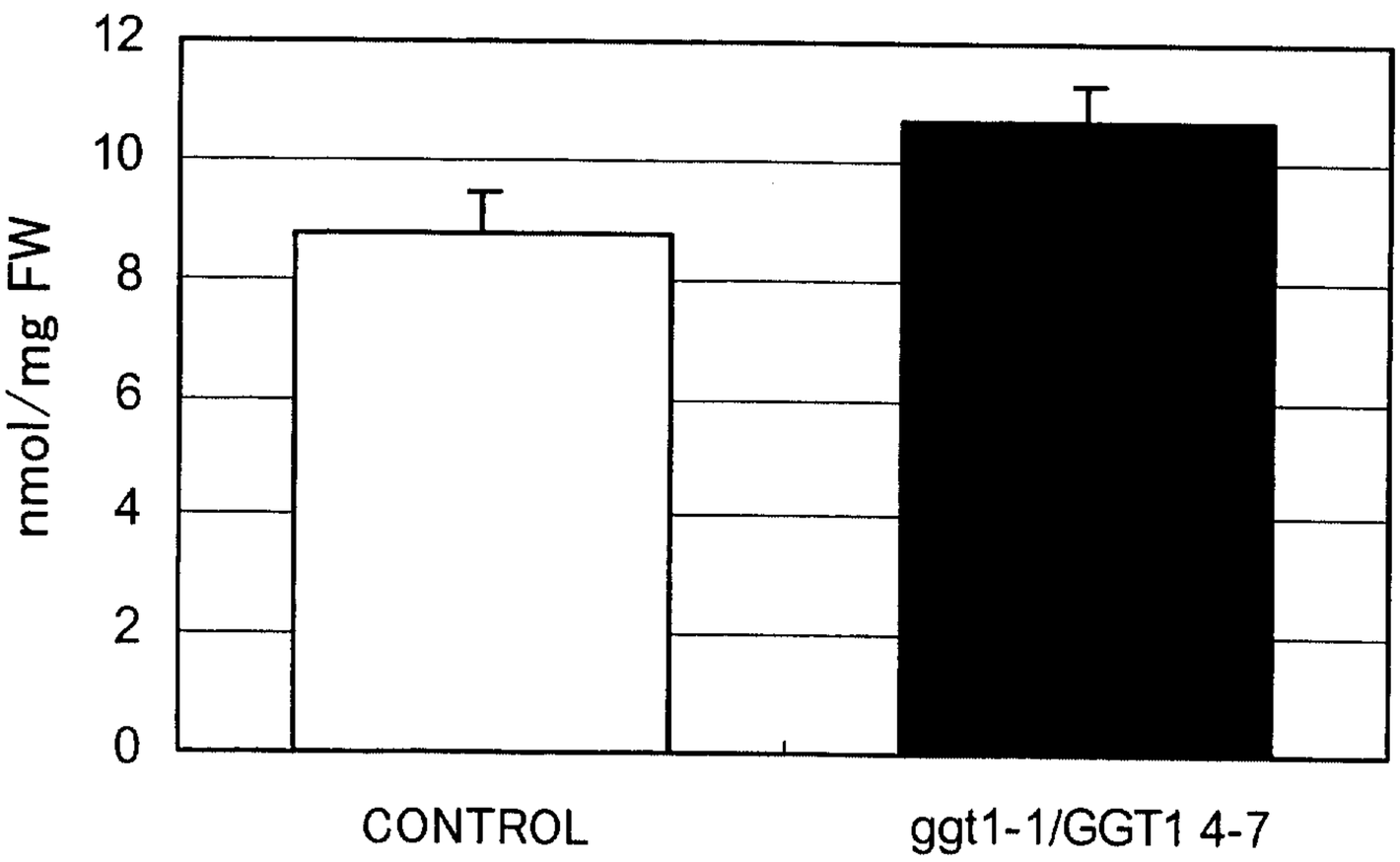
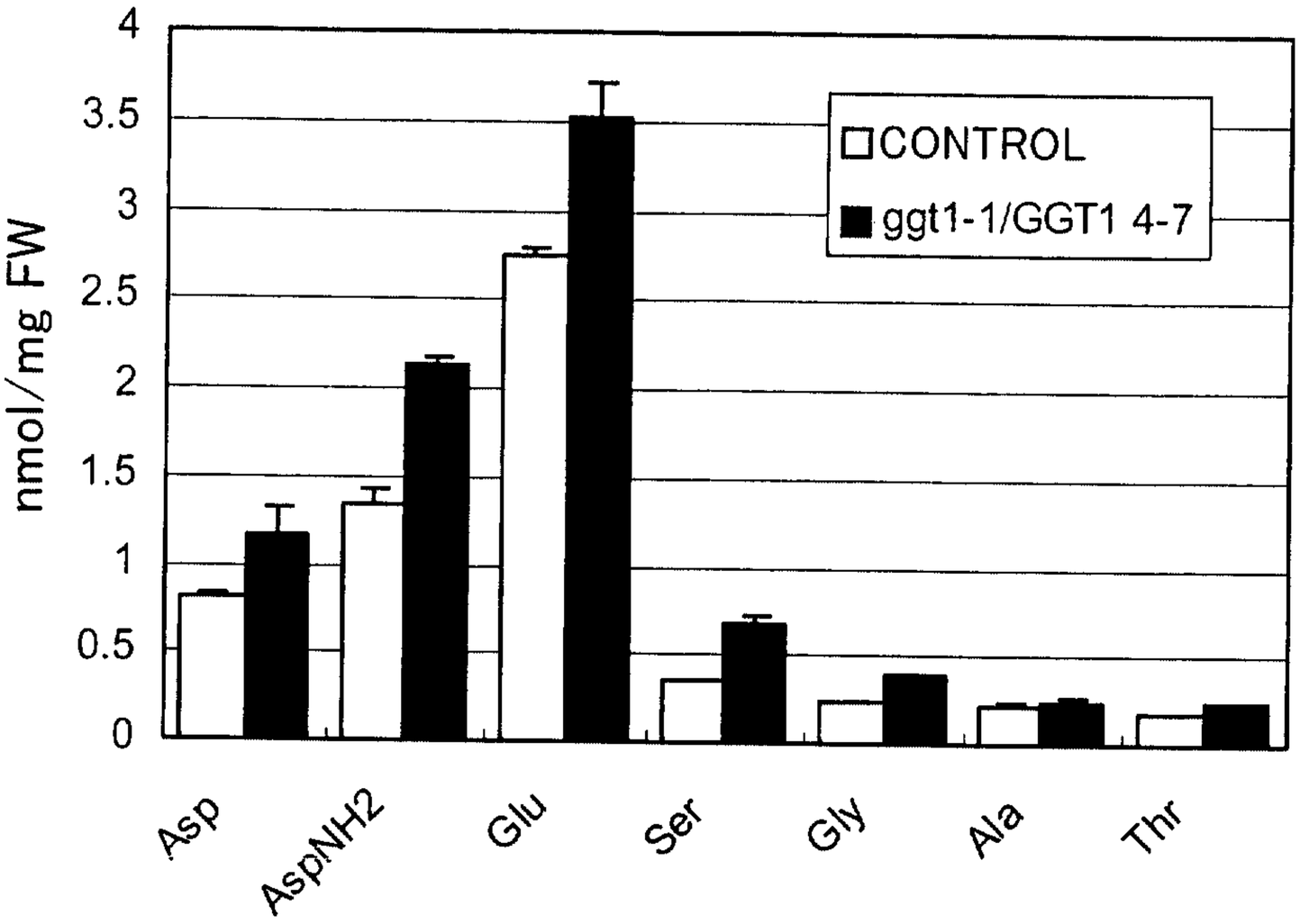
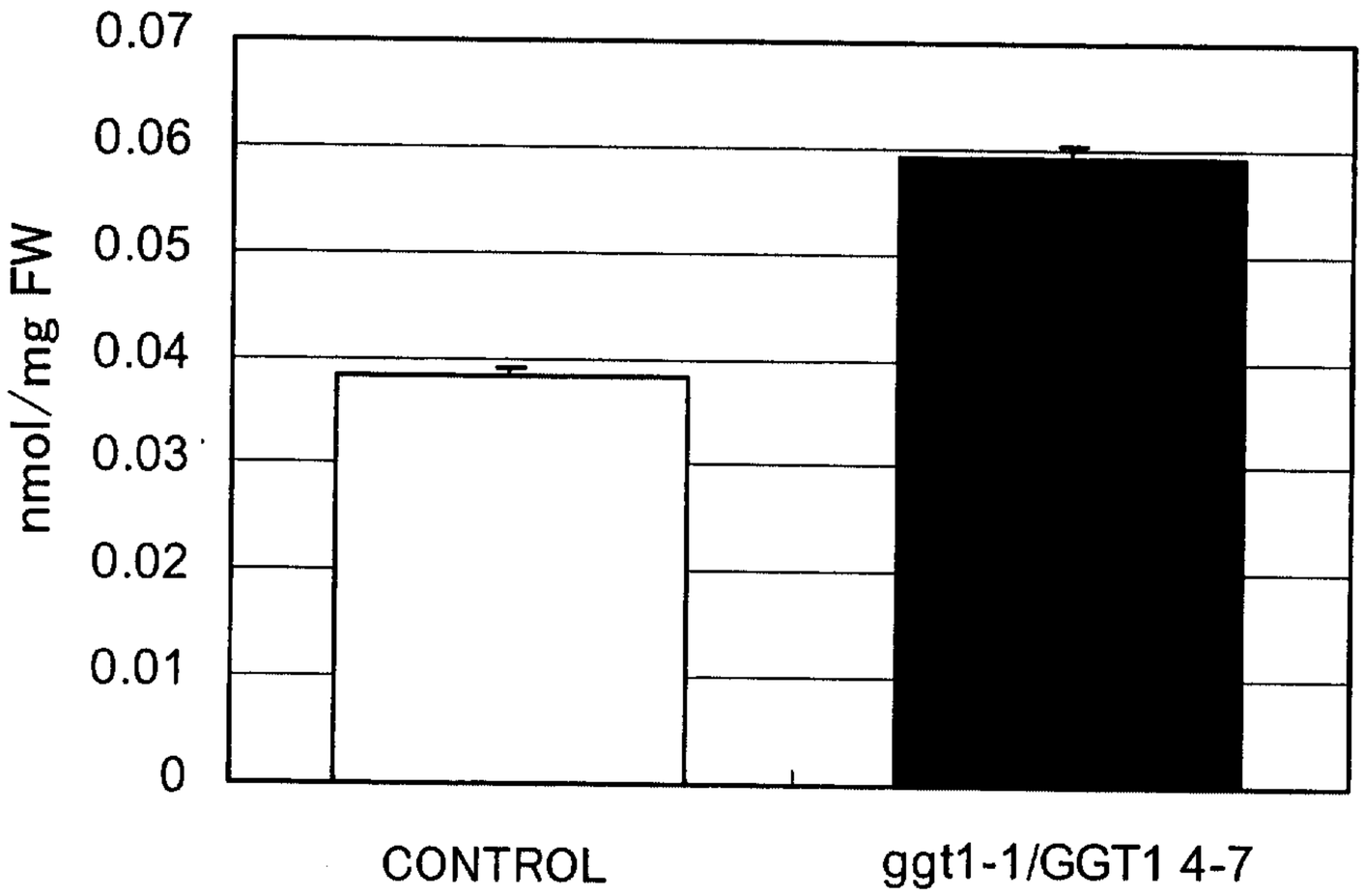

nmol/mg FW
12
10
8
6
4
2
0
CONTROL
ggt1-1/GGT1 4-7

FIG. 15

(A)


nmol/mg FW
4
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
ggt1-1/GGT1 4-7
Asp
AspNH2
Glu
Ser
Gly
Ala
Thr

(B)


nmol/mg FW
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
CONTROL
ggt1-1/GGT1 4-7

(C)

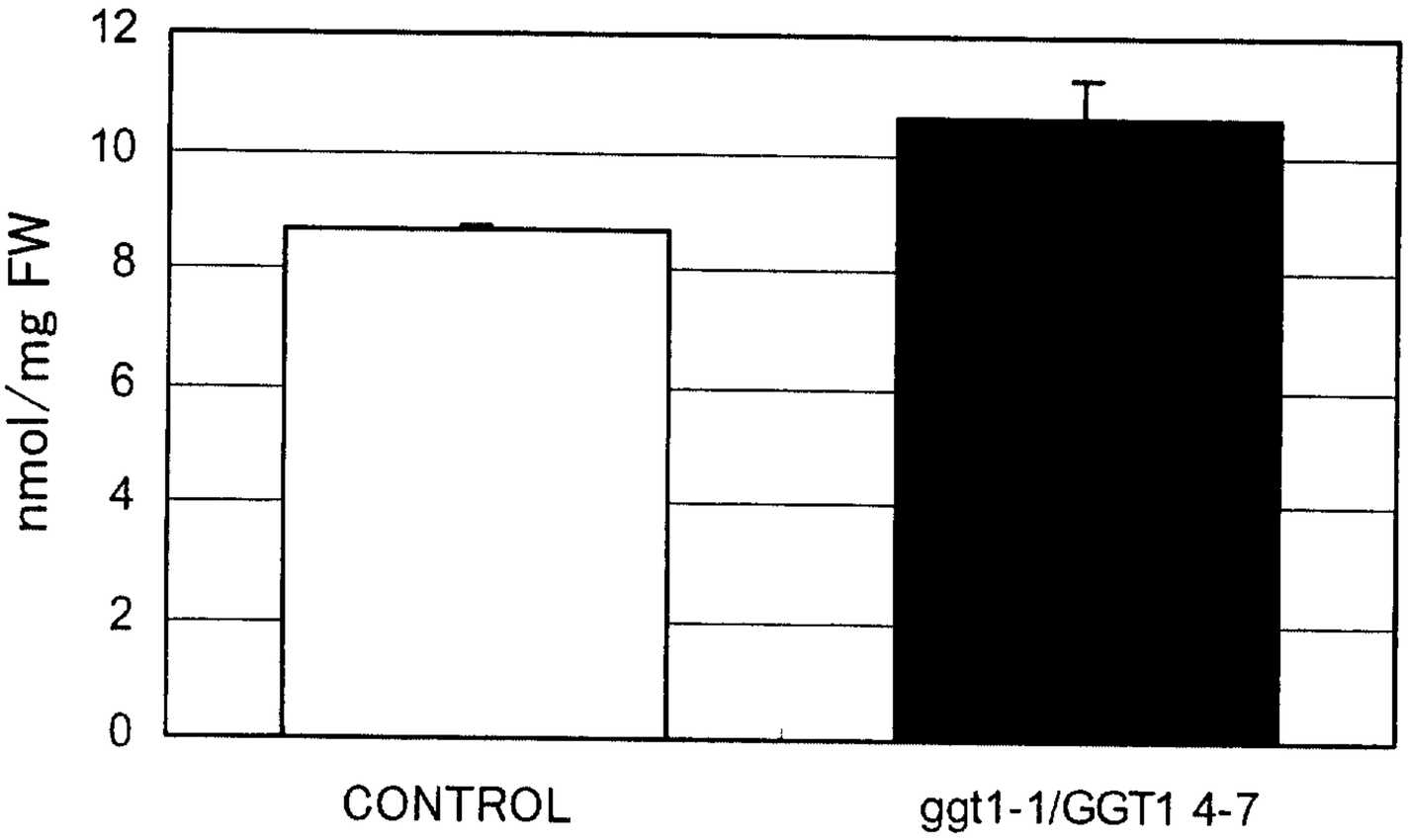
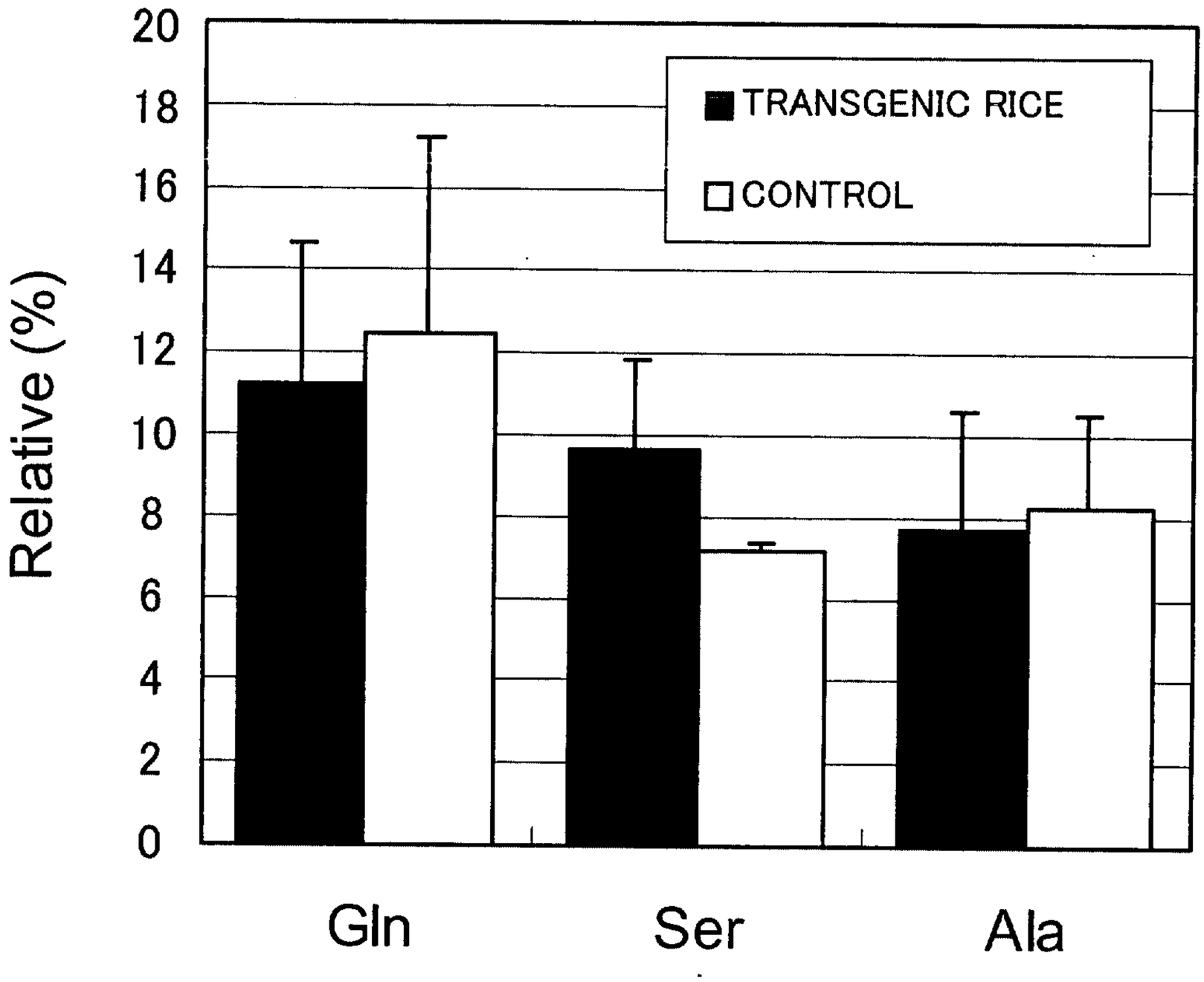

nmol/mg FW
12
10
8
6
4
2
0
CONTROL
ggt1-1/GGT1 4-7

FIG. 16

```
GGT1            -------MALKALDYDTLNENVKKCQYAVRGELYLRASELQKEGKKIIFTNVGNPHALGQ
Japonica_GGT    MFGGGGGGGRKPLDYEELNENVKKVQYAVRGELYLRASELQKEGKKIIFTNVGNPHALGQ
Indica_GGT      -MFGGGGGGRKPLDYEELNENVKKVQYAVRGELYLRASELQKEGKKIIFTNVGNPHALGQ
                        * ***   ******* ********************************

GGT1            KPLTFPRQVVALCQAPFLLDDPNVGMLFPADAIARAKHYLSLTSGGLGAYSDSRGLPGVR
Japonica_GGT    KPLTFPRQVVALCQAPFLLDDPNVGLIFPADAIARAKHYLAMAPG-GLAYSDSRGIPGIR
Indica_GGT      KPLTFPRQVVALCQAPFLLDDPNVGLIFPADAIARAKHYLAMAPGGLGAYSDSRGIPGIR
                ************************  **************     *    ******* ** *

GGT1            KEVAEFIQRRDGYPSDPELIFLTDGASKGVMQILNCVIRGNGDGILVPVPQYPLYSATIS
Japonica_GGT    KEVAEFIERRDGYP-DPELIYLTDGASKGVMQMLNTIIRNERDGILVPVPQYPLYSAAIS
Indica_GGT      KEVAEFIERRDGYPSDPELIYLTDGASKGVMQMLNTIIRNERDGILVPVPQYPLYSAAIS
                ******* ****** ***** ********** **   **    ********************

GGT1            LLGGTLVPYYLDESENWGLDVANLRQSVAQARSQGITVRAMVIINPGNPTGQCLSEANIR
Japonica_GGT    LFGGSLVPYYLEEEANWGLDFVNLRQTVASARSKGITVRAMVIINPGNPTGQCLSEGNIK
Indica_GGT      PFGGSLVPYYLEEEANWGLDFVNLRQTVASARSKGITVRAMVIINPGNPTGQCLSEGNIK
                  ** ****** *   *****  **** ** *** ************************ **

GGT1            EILKFCYNEKLVLLGDEVYQQNIYQDERPFISSKKVLMEMGSPFSKEVQLVSFHTVSKGY
Japonica_GGT    ELLKFCFHENLVLLADEVYQQNIYQDERPFISARKVLFDMGPPMSREVQLVSFHTVSKGY
Indica_GGT      ELLKFCFHENLVLLADEVYQQNIYQDERPFISARKVLFDMGPPMSREVQLVSFHTVSKGY
                * ****  * **** ******************  ***  ** * * **************

GGT1            WGECGQRGGYFEMTNLPPRVVEEIYKVASIALSPNVSAQIFMGLMVNPPKPGDISYDQFA
Japonica_GGT    WGECGQRGGYFEMTNLPPKTVDEIYKVASIALSPNVPGQIFMGLMVNPPKPGDISYLKFS
Indica_GGT      WGECGQRGGYFEMTNLPPKTVDEIYKVASIALSPNVPGQIFMGLMVNPPKPGDISYLKFS
                ********************  * ***************  ********************  *

GGT1            RESKGILESLRRRARLMTDGFNSCKNVVCNFTEGAMYSFPQIRLPTGALQAAKQAGKVPD
Japonica_GGT    AEKS-ILESLRRRARLMTDGFNSCRNVVCNFTE-AMYSFPQIRLPPKAIDAAKRAGKAAD
Indica_GGT      AESKSILESLRRRARLMTDGFNSCRNVVCNFTEGAMYSFPQIRLPPKAIDAAKRAGKAAD
                 *    ********************** ******** ***********  *  *** ***  *

GGT1            VFYCLKLLEATGISTVPGSGFGQKEGVFHLRTTILPAEDEMPEIMDSFKKFNDEFMTQYD
Japonica_GGT    VFYCLKLLEATGISTVPGSGFGQKE-VFHLRTTILPAEEDMPAIMTSFKKFNDTFMDQYD
Indica_GGT      VFYCLKLLEATGISTVPGSGFGQKEGVFHLRTTILPAEEDMPAIMTSFKKFNDTFMDQYD
                ***************************** ************  ** ** ******* ** ***

GGT1            NNFGYSKM
Japonica_GGT    ---GYSRM
Indica_GGT      ---GYSRM
                   *** *
```

FIG. 17


20
18
16
14
12
10
8
6
4
2
0
Relative (%)
TRANSGENIC RICE
CONTROL
Gln
Ser
Ala