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(54) **TRANSGENIC PLANTS WITH ENHANCED GROWTH CHARACTERISTICS**

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(52) **U.S. Cl.** **800/278**; 435/419; 800/298; 800/305; 800/306; 800/307; 800/309; 800/310; 800/313; 800/314; 800/315; 800/316; 800/317; 800/320; 800/321; 800/323; 800/323.1; 800/323.2; 800/323.3

(57) **ABSTRACT**

The invention relates to transgenic plants exhibiting dramatically enhanced growth rates, greater seed and fruit/pod yields, earlier and more productive flowering, more efficient nitrogen utilization, increased tolerance to high salt conditions, and increased biomass yields. In one embodiment, transgenic plants engineered to over-express both glutamine phenylpyruvate transaminase (GPT) and glutamine synthetase (GS) are provided. The GPT+GS double-transgenic plants of the invention consistently exhibit enhanced growth characteristics, with T0 generation lines showing an increase in biomass over wild type counterparts of between 50% and 300%. Generations that result from sexual crosses and/or selfing typically perform even better, with some of the double-transgenic plants achieving an astounding four-fold biomass increase over wild type plants.

FIG. 1

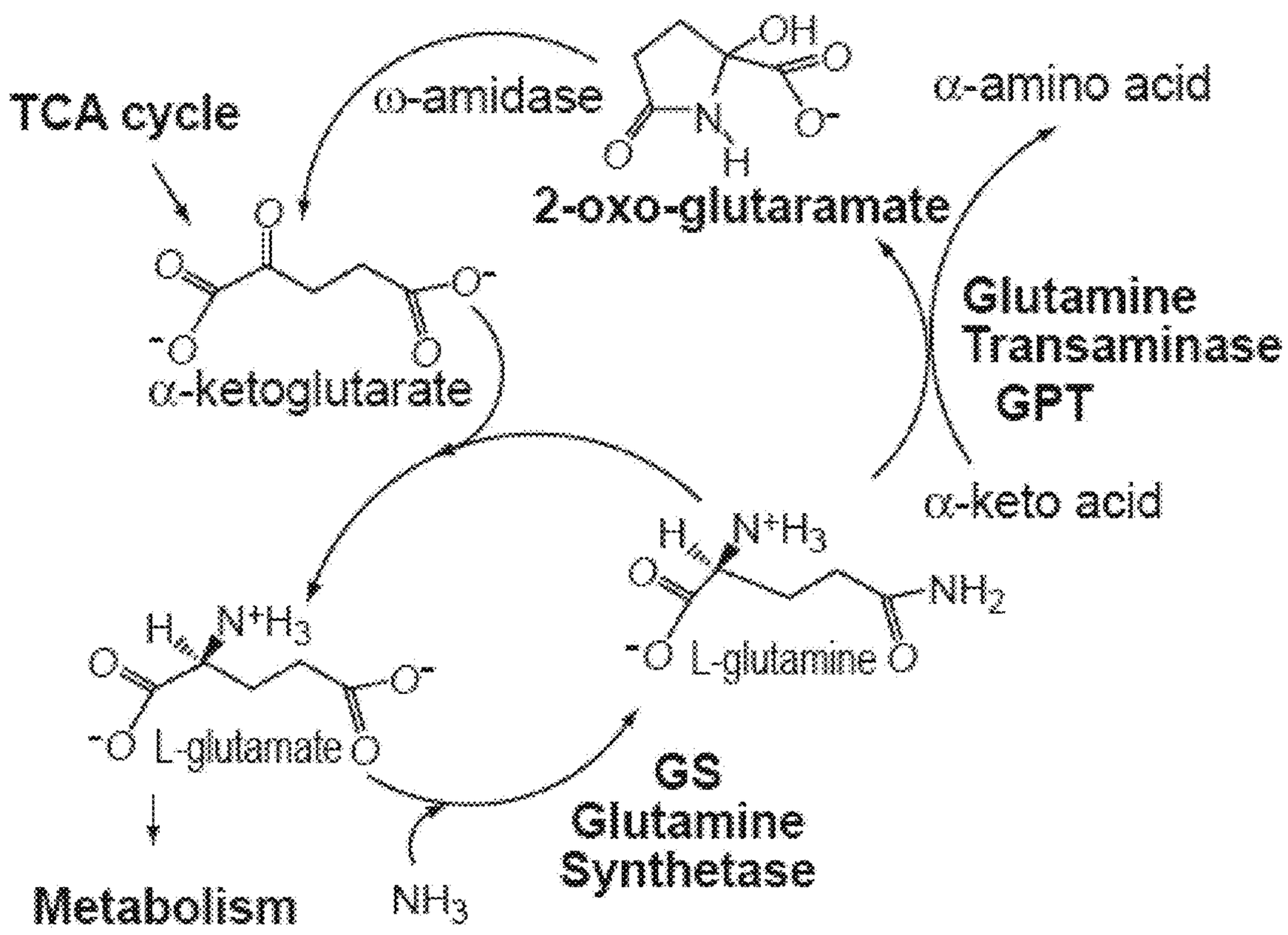

TCA cycle
ω-amidase
α-amino acid
2-oxo-glutaramate
α-ketoglutarate
Glutamine
Transaminase
GPT
α-keto acid
L-glutamine
L-glutamate
GS
Glutamine
Synthetase
Metabolism
NH3

FIG. 2

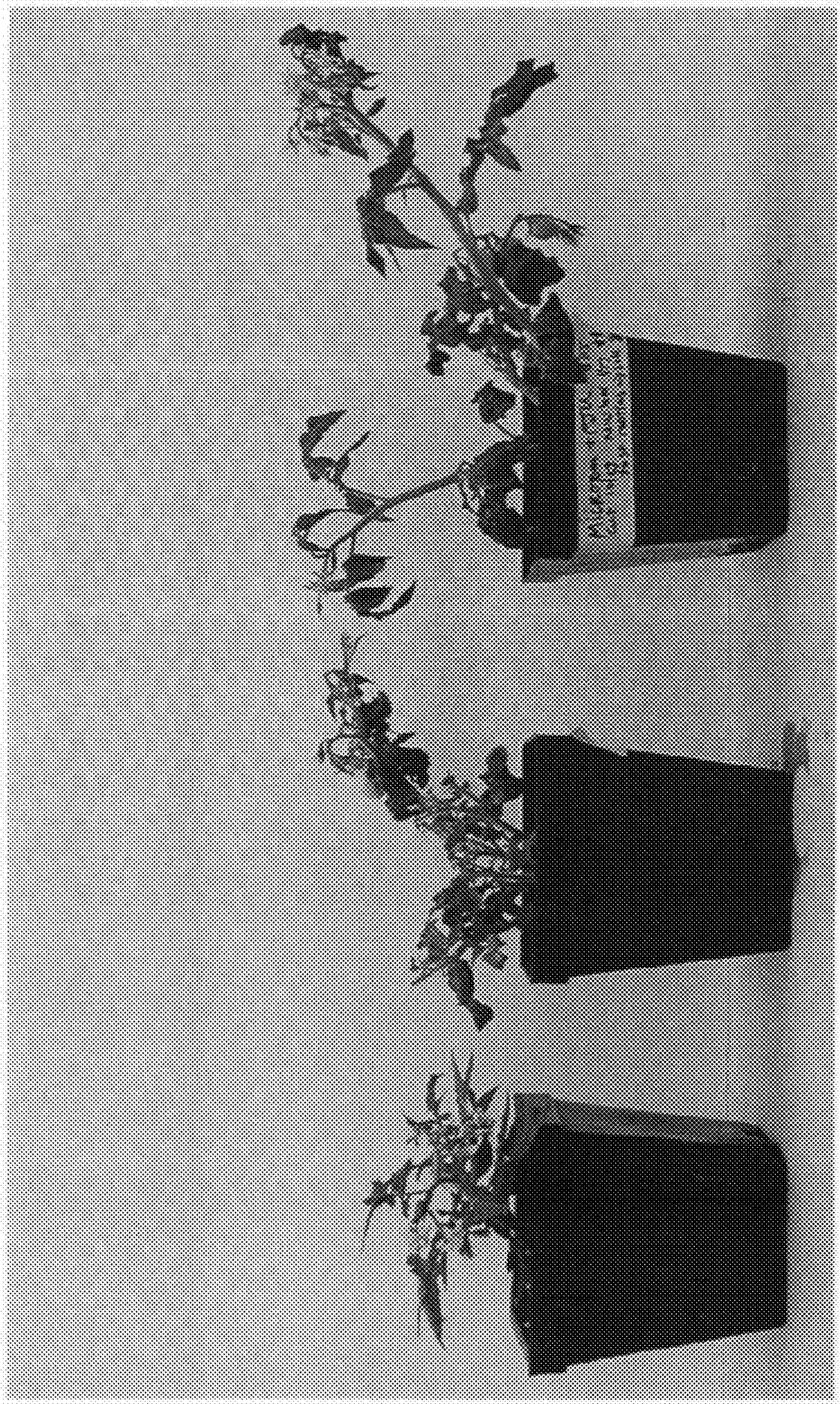


FIG. 3

FIG. 4A

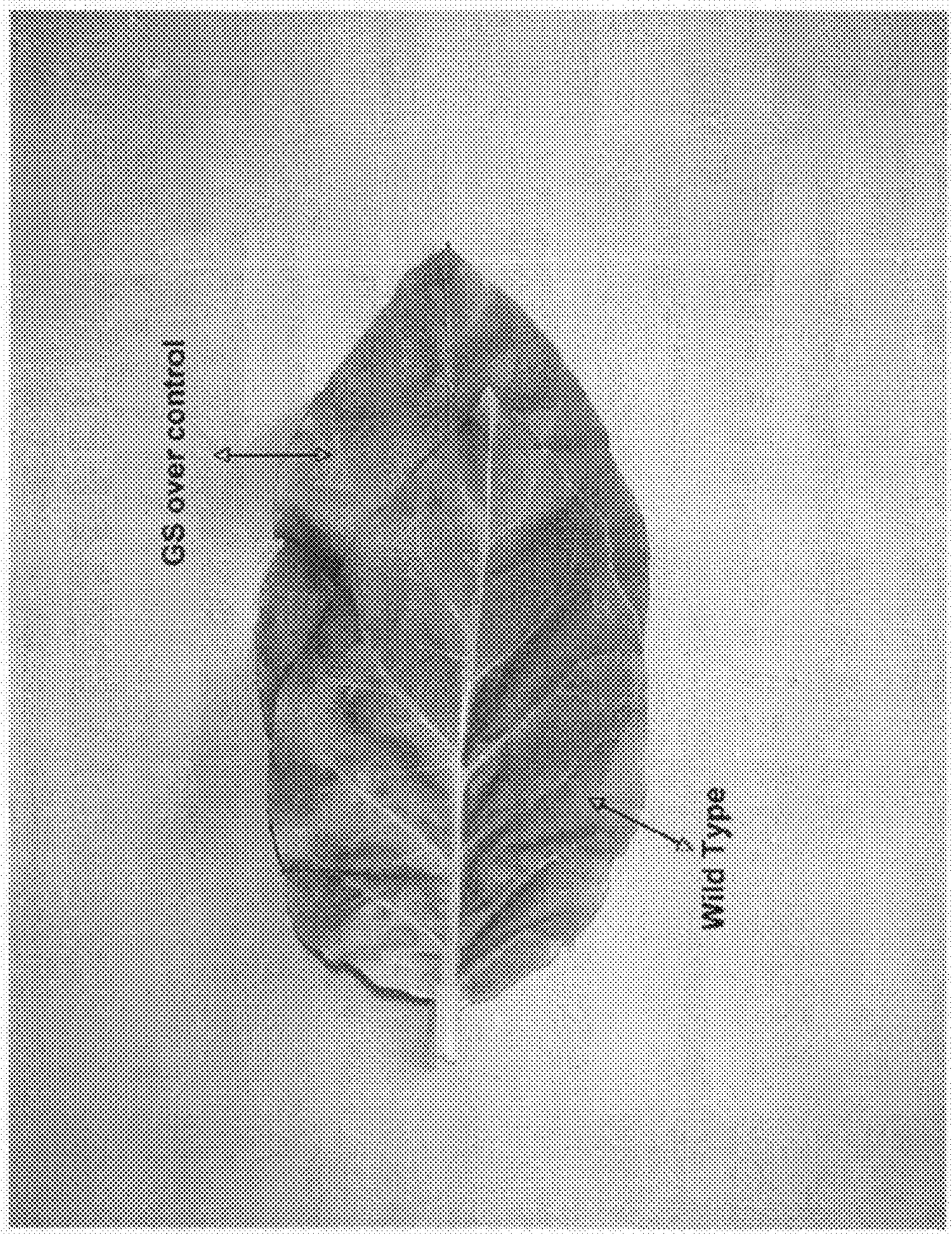


FIG. 4B

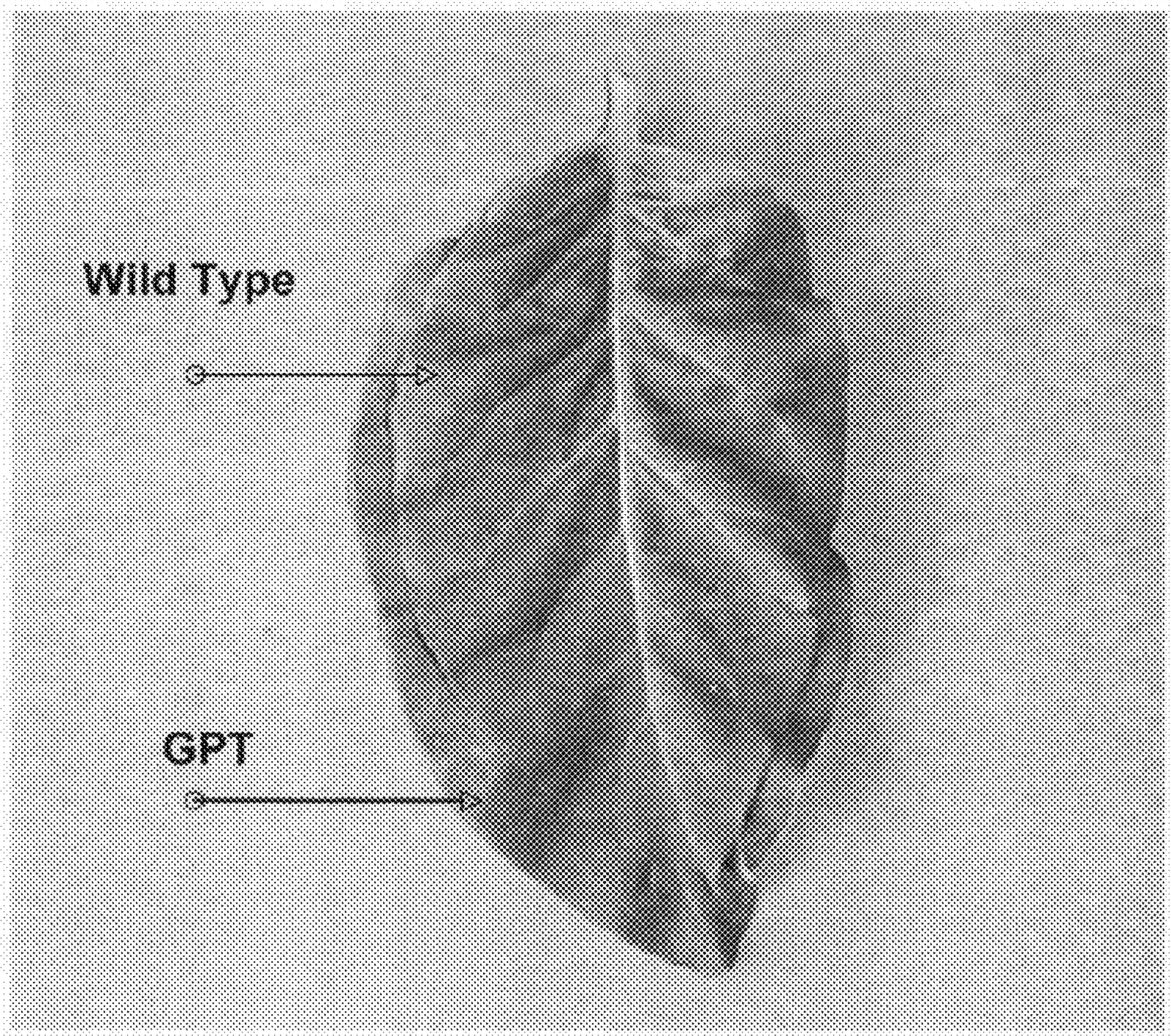


FIG. 5A

FIG. 5B

FIG. 5C

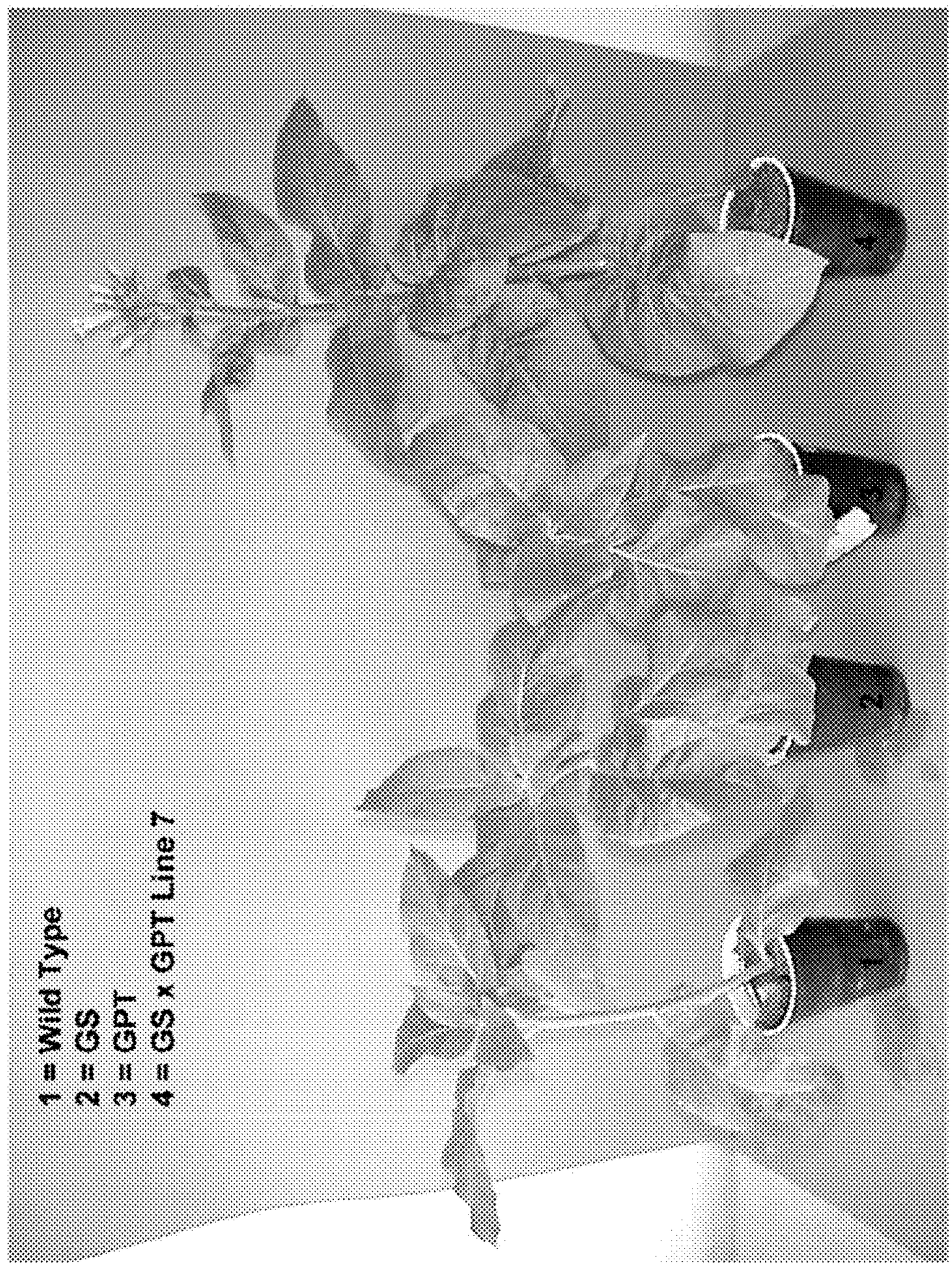


FIG. 6A

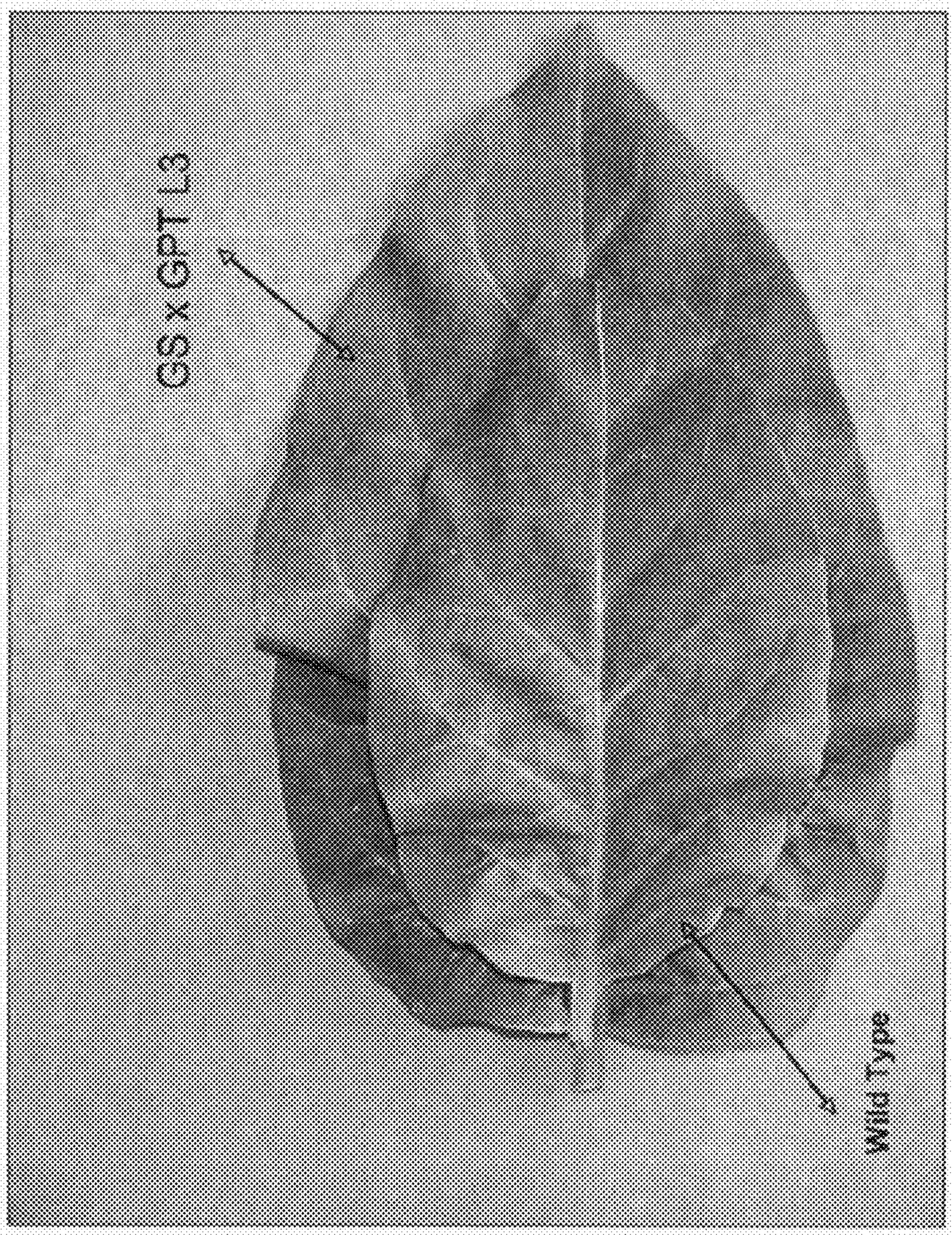


FIG. 6B

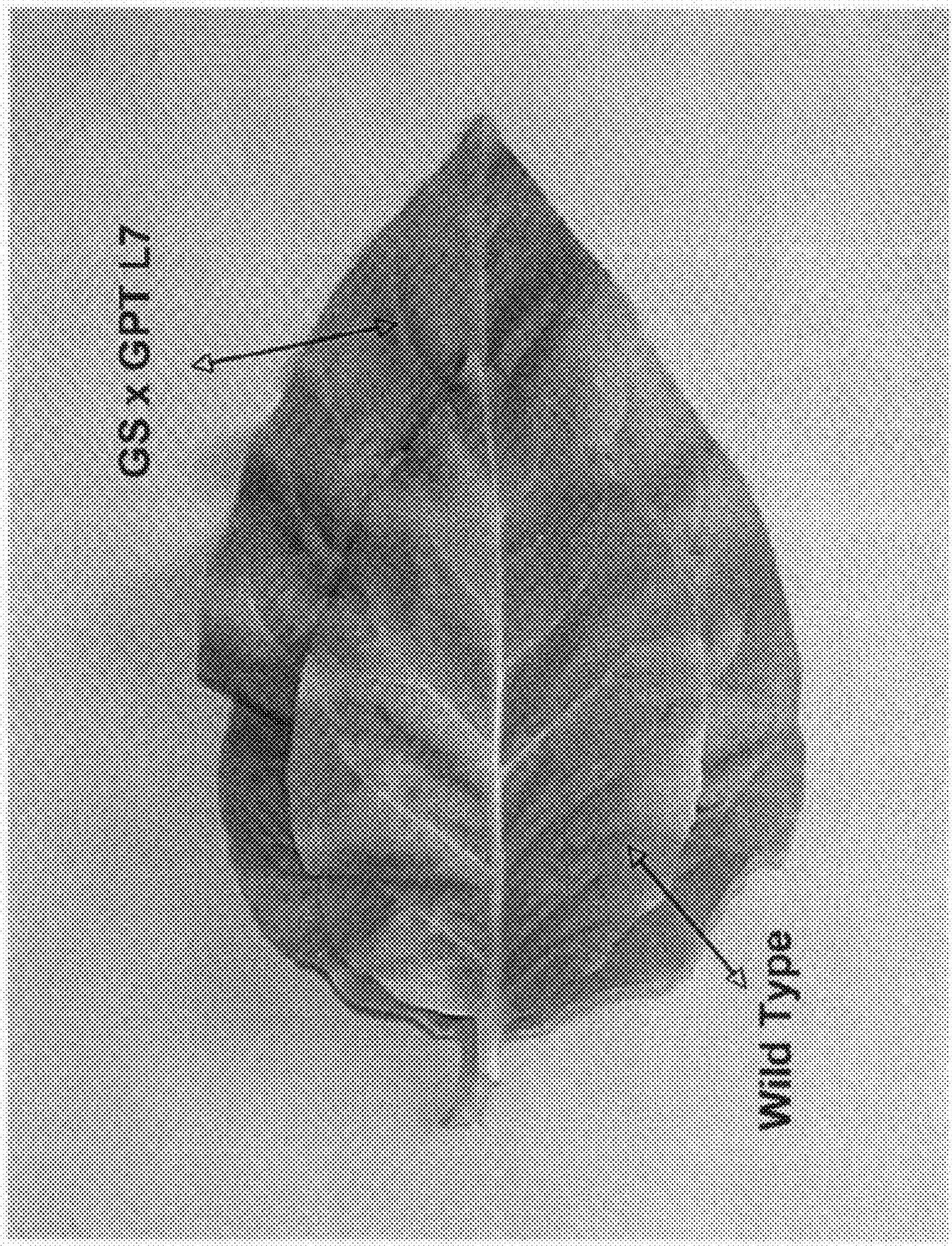
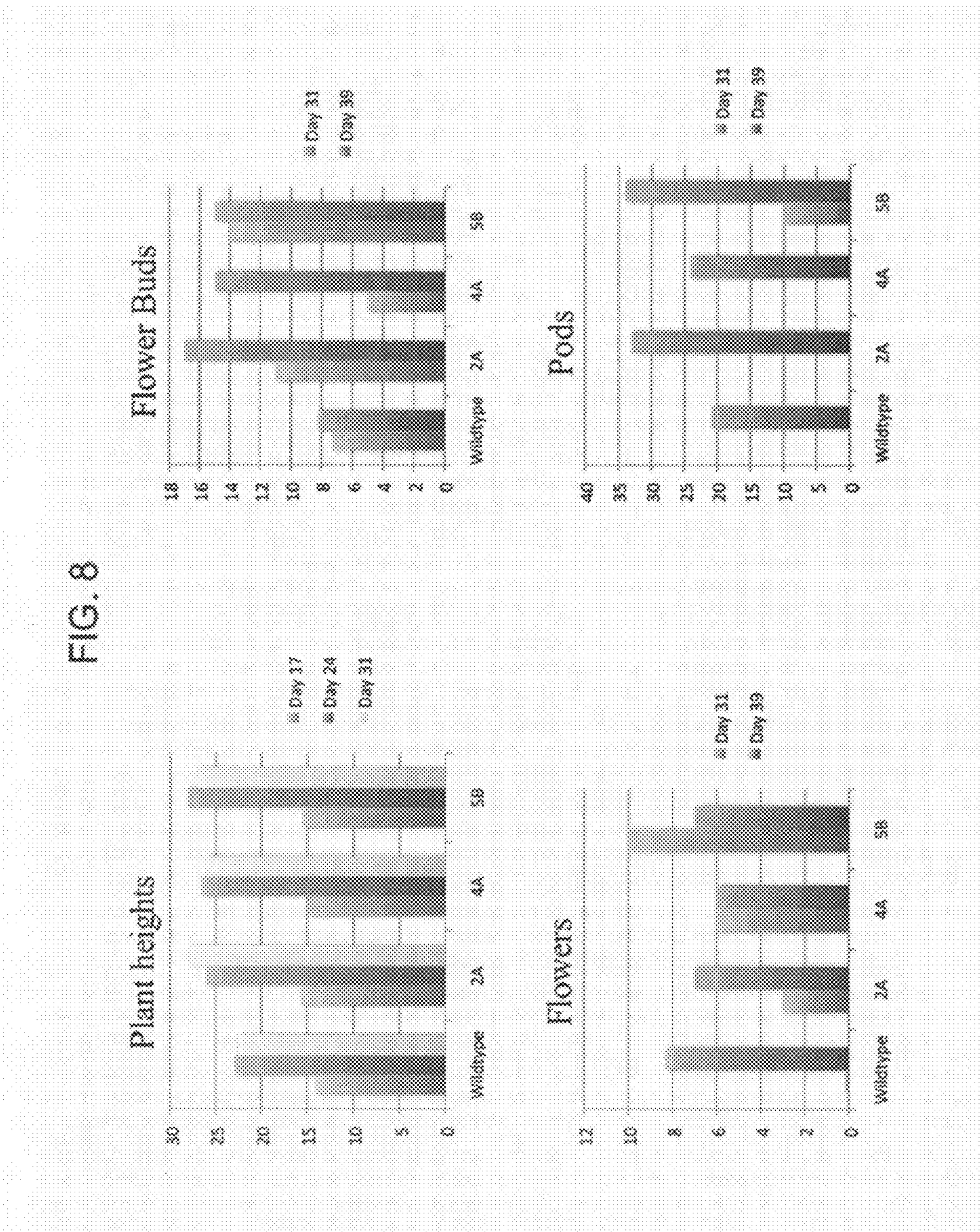


FIG. 7

FIG. 8


Plant heights
30
25
20
15
10
5
0
Wildtype
2A
4A
5B
Day 17
Day 24
Day 31
Flowers
12
10
8
6
4
2
0
Wildtype
2A
4A
5B
Day 31
Day 39
Flower Buds
18
16
14
12
10
8
6
4
2
0
Wildtype
2A
4A
5B
Day 31
Day 39
Pods
40
35
30
25
20
15
10
5
0
Wildtype
2A
4A
5B
Day 31
Day 39

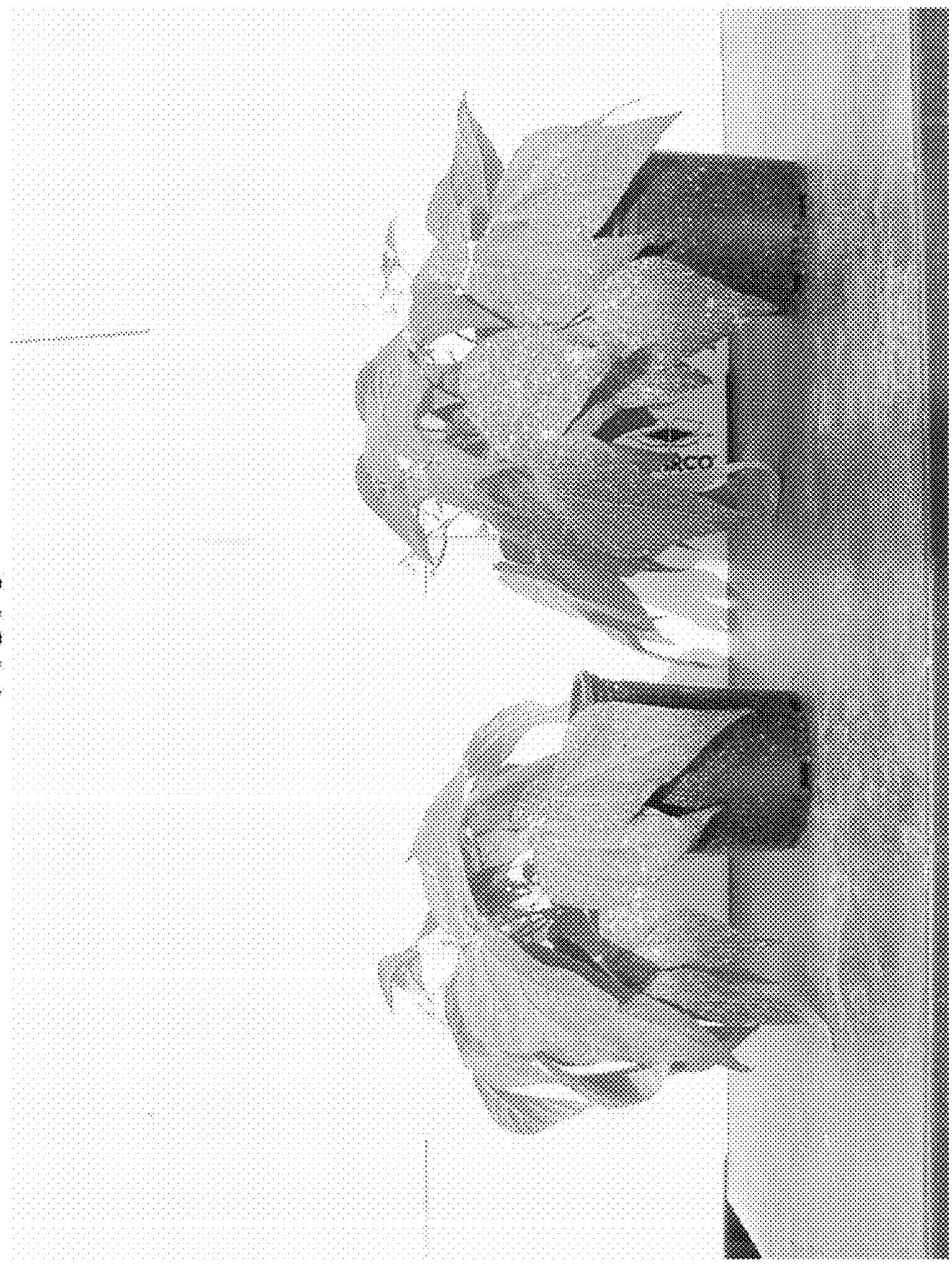


FIG. 9

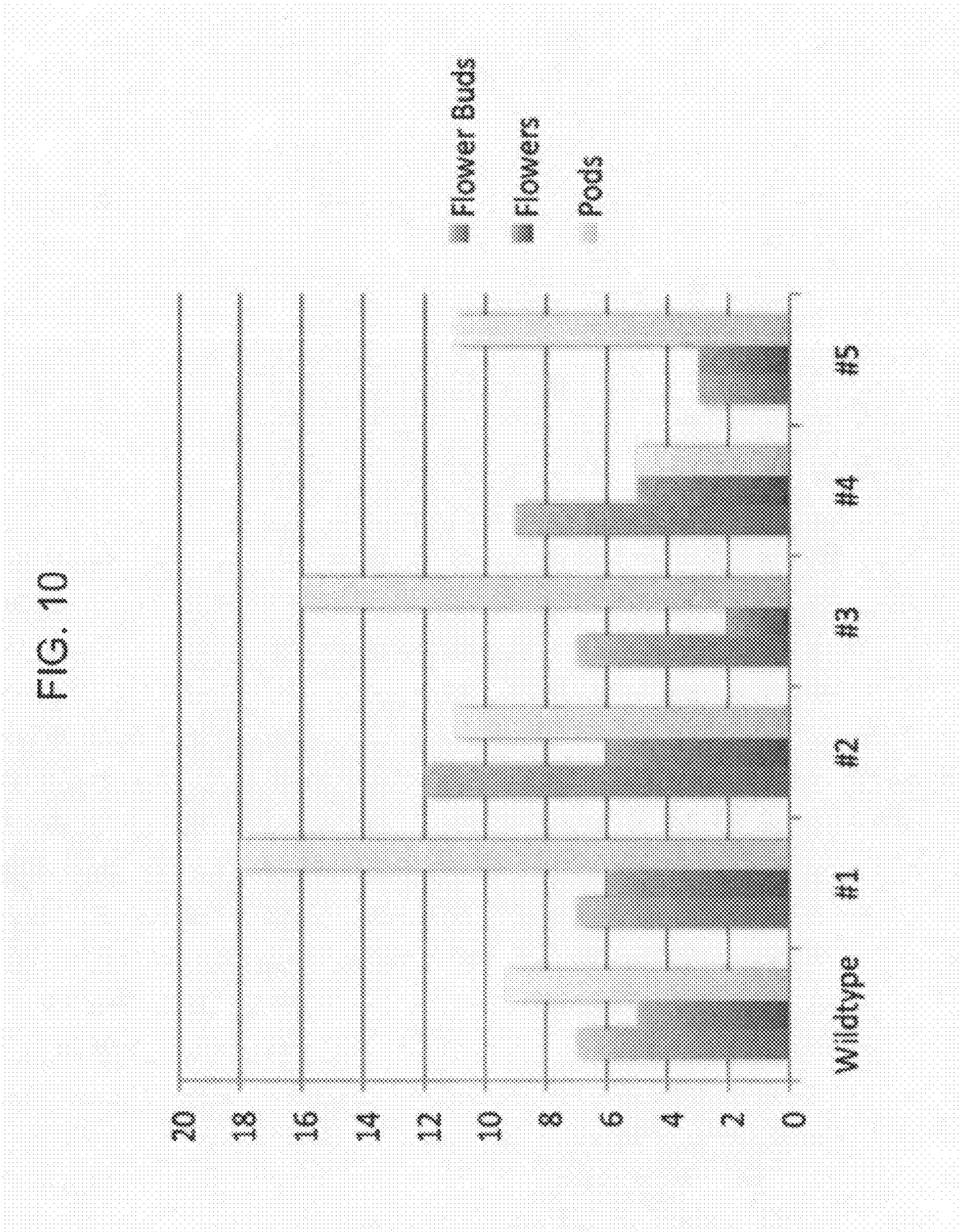
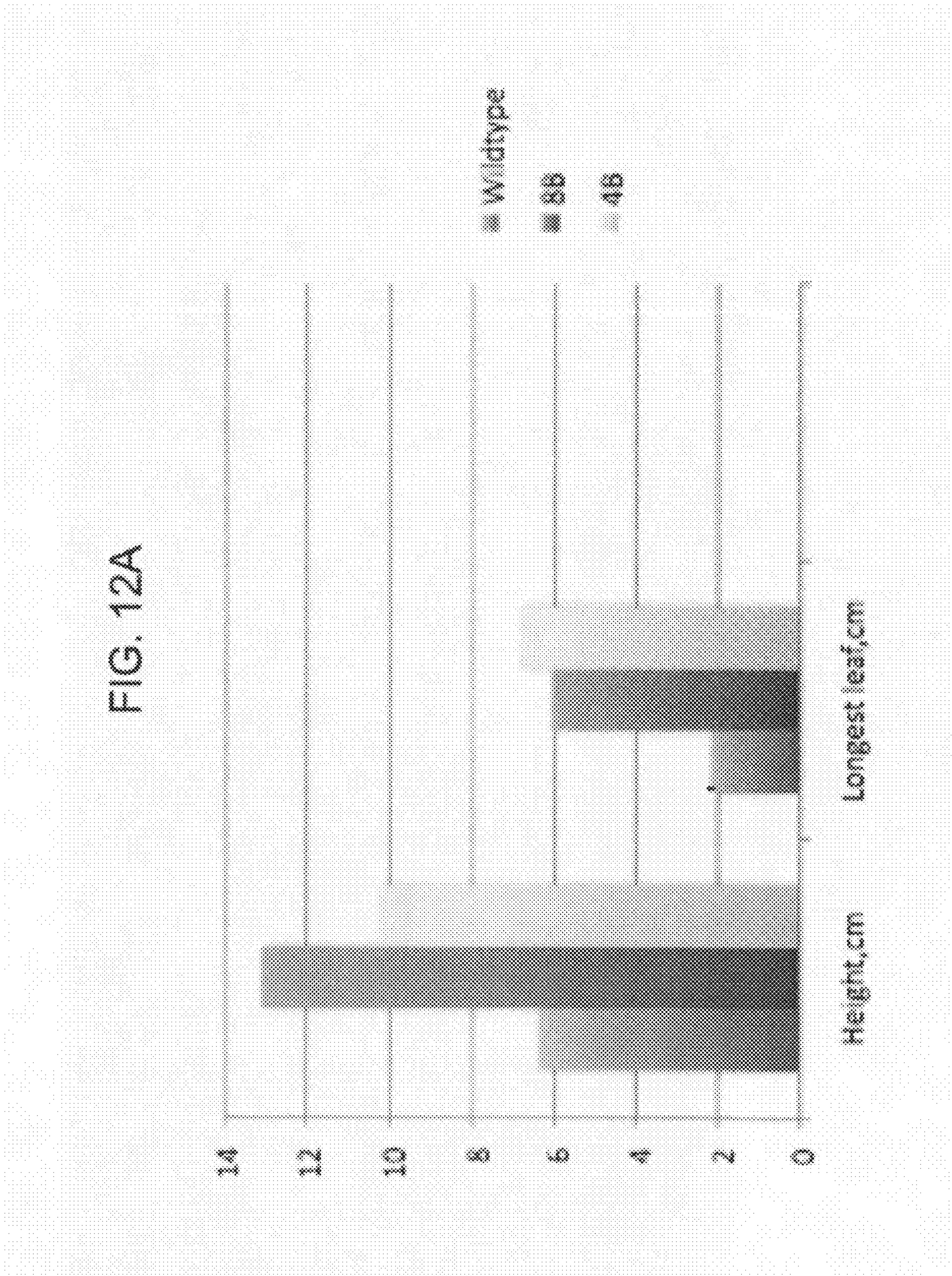

Flower Buds
Flowers
Pods
20
18
16
14
12
10
8
6
4
2
0
Wildtype
#1
#2
#3
#4
#5

FIG. 10

FIG. 11

FIG. 12A



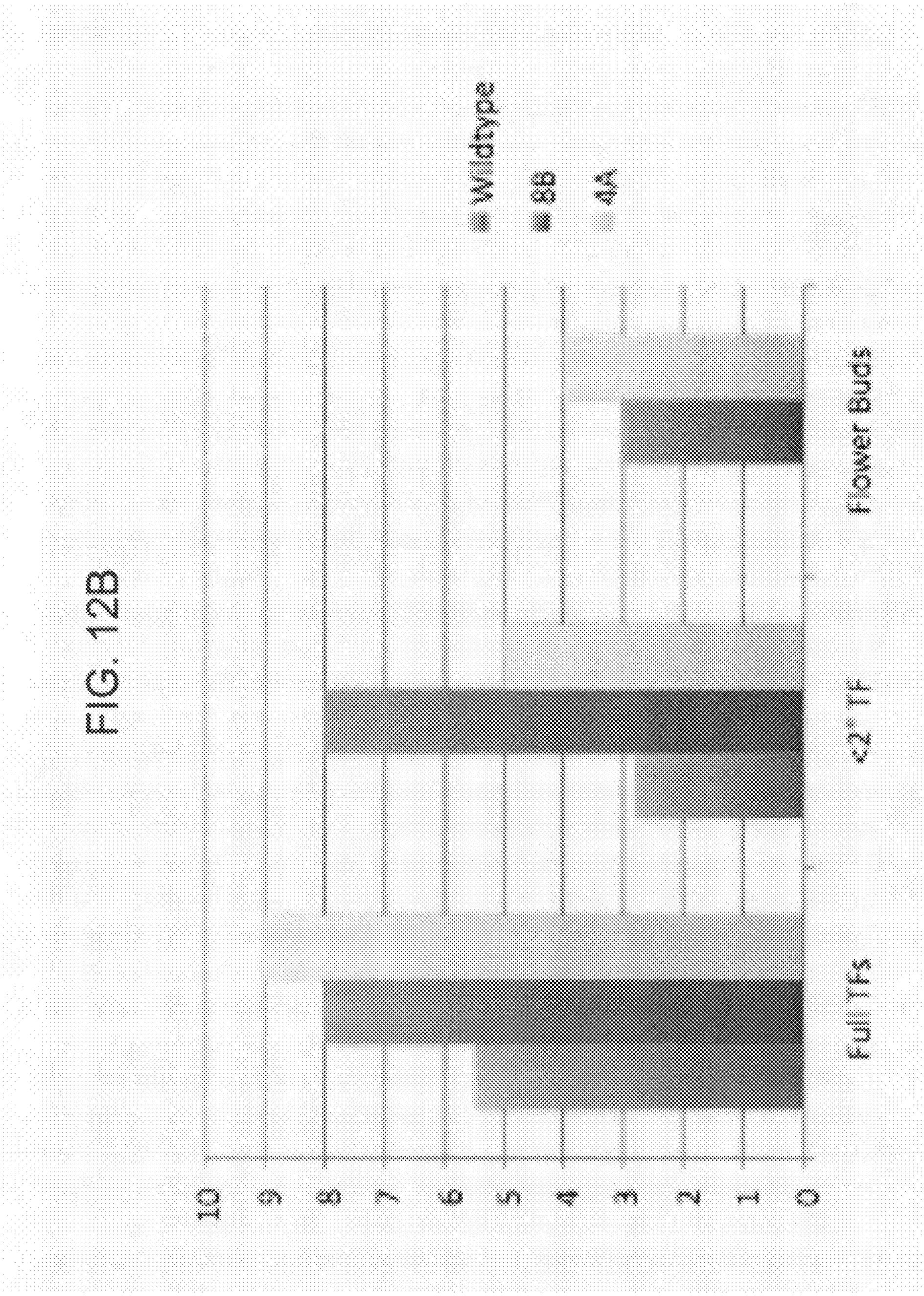


FIG. 12B
wt/type
8B
4A
10
9
8
7
6
5
4
3
2
1
0
Full TFs
<2° TF
Flower Buds

FIG. 12C

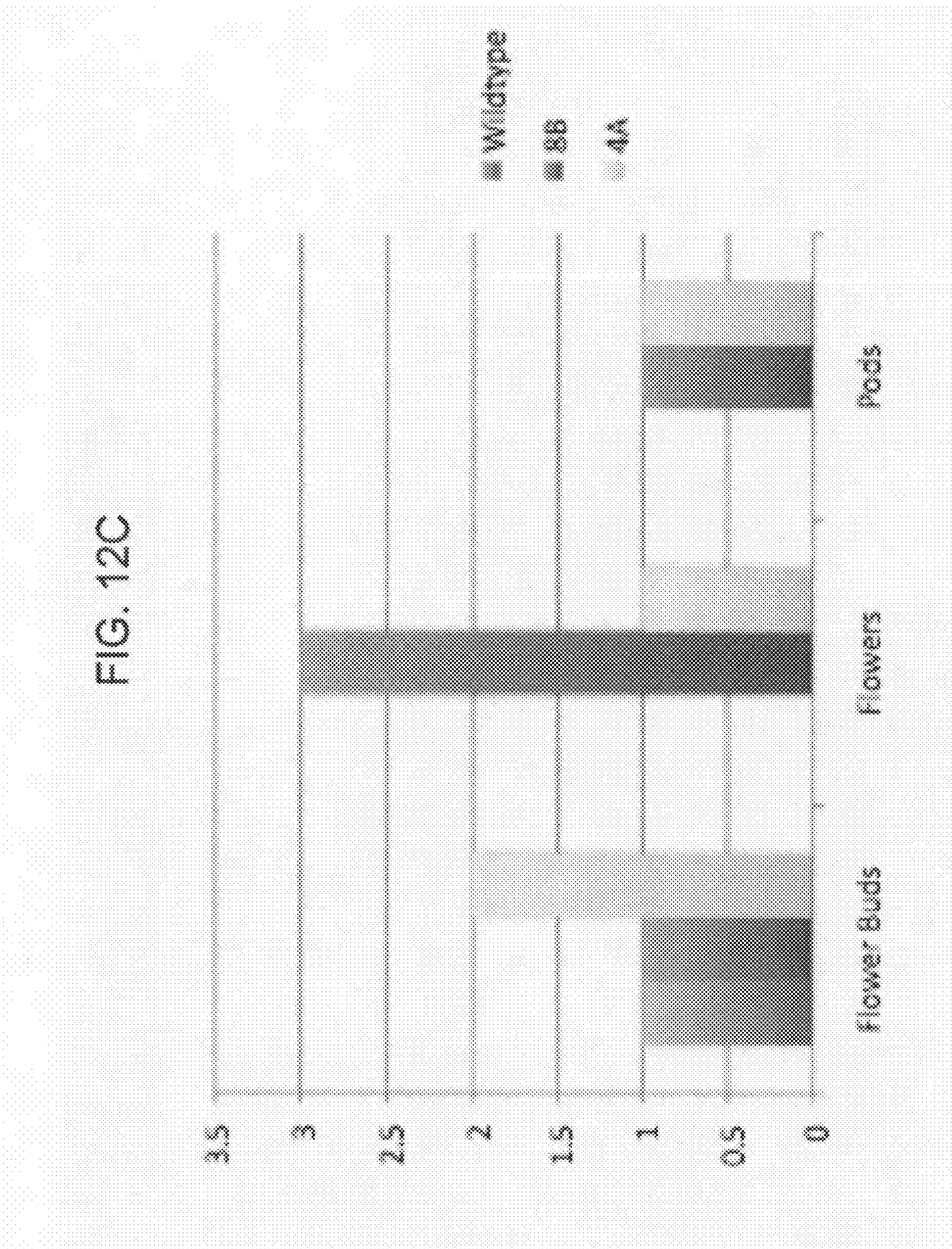


FIG. 13

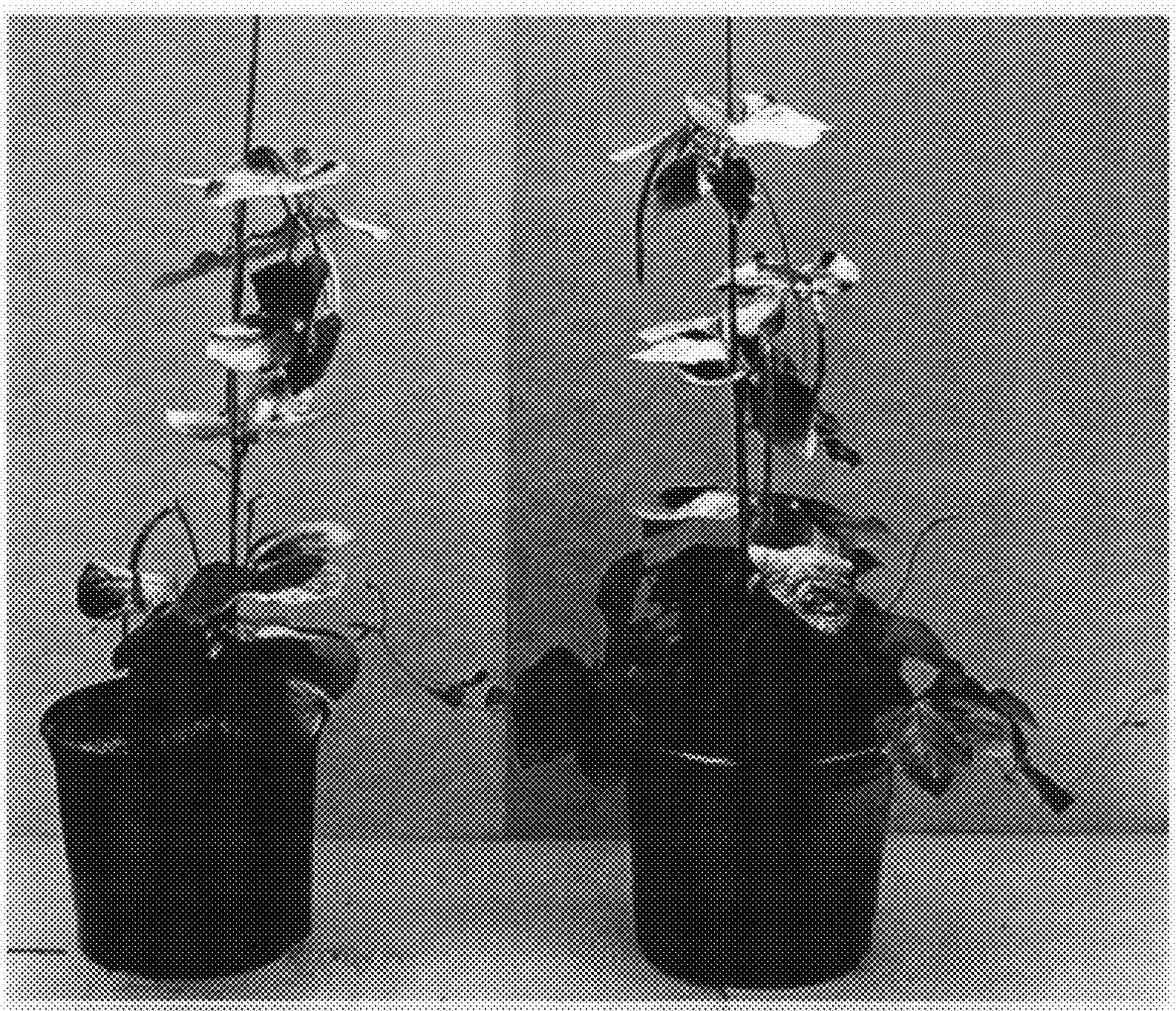


FIG. 14A

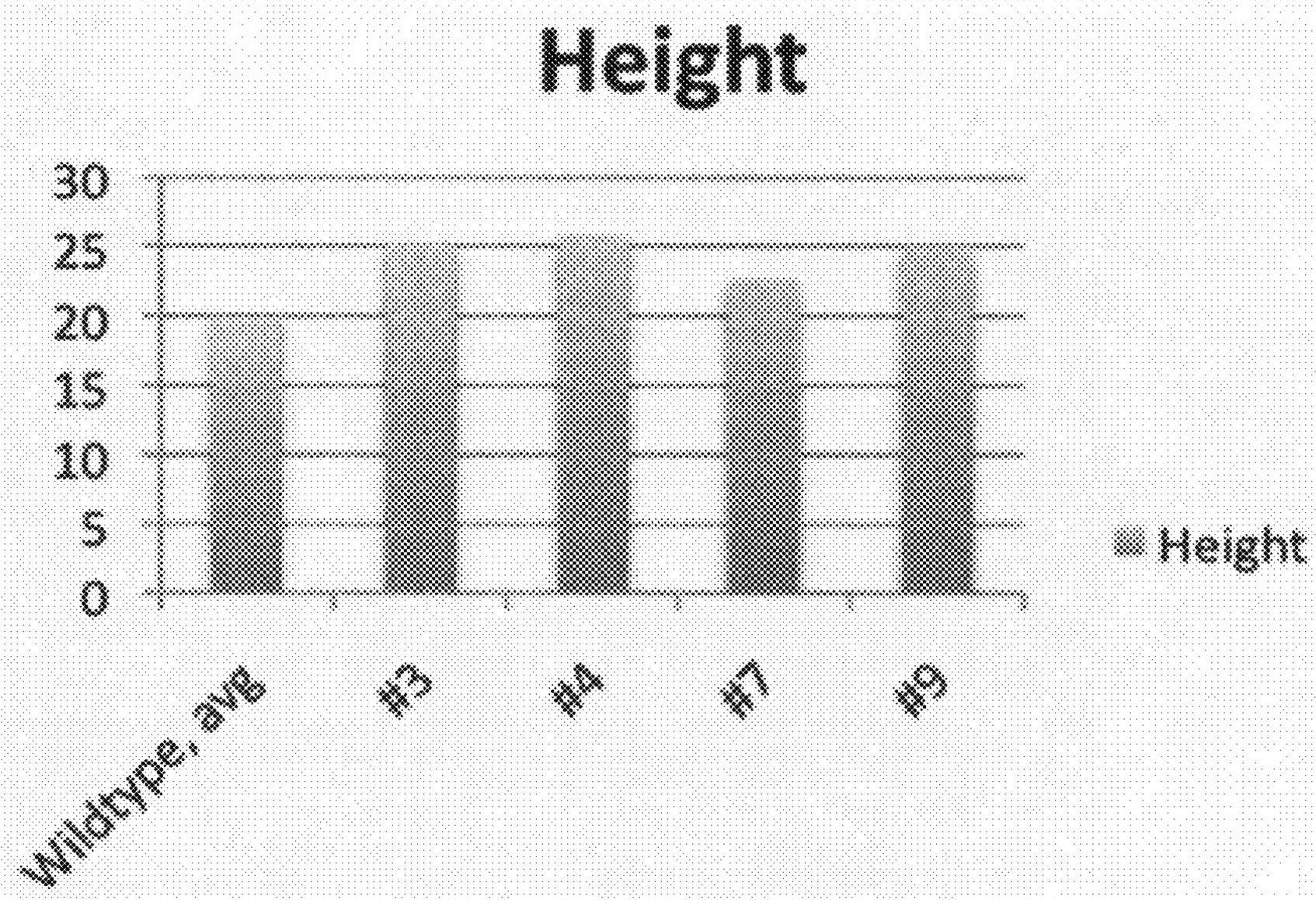
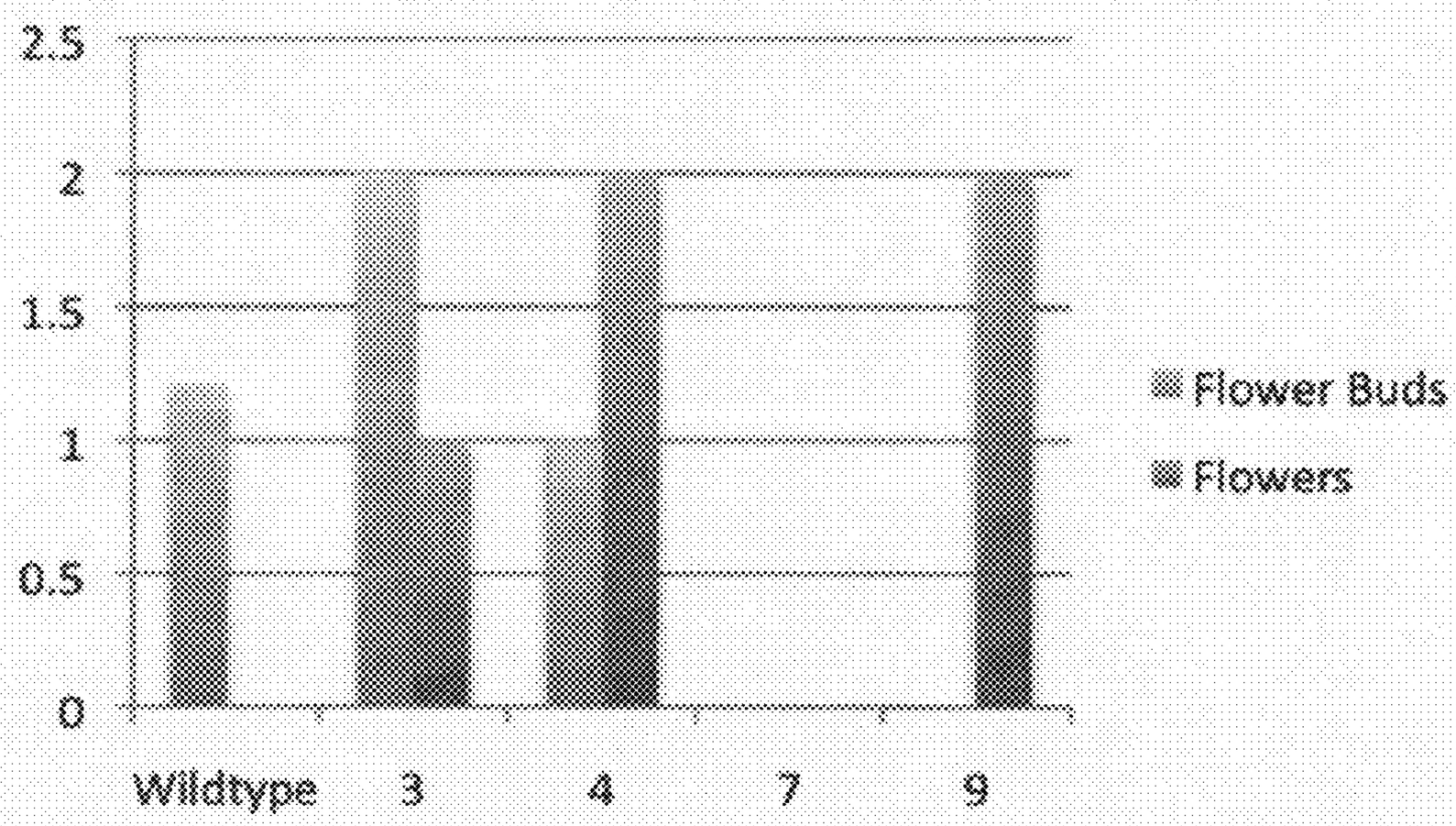


FIG. 14B


2.5
2
1.5
1
0.5
0
Wildtype
3
4
7
9
Flower Buds
Flowers

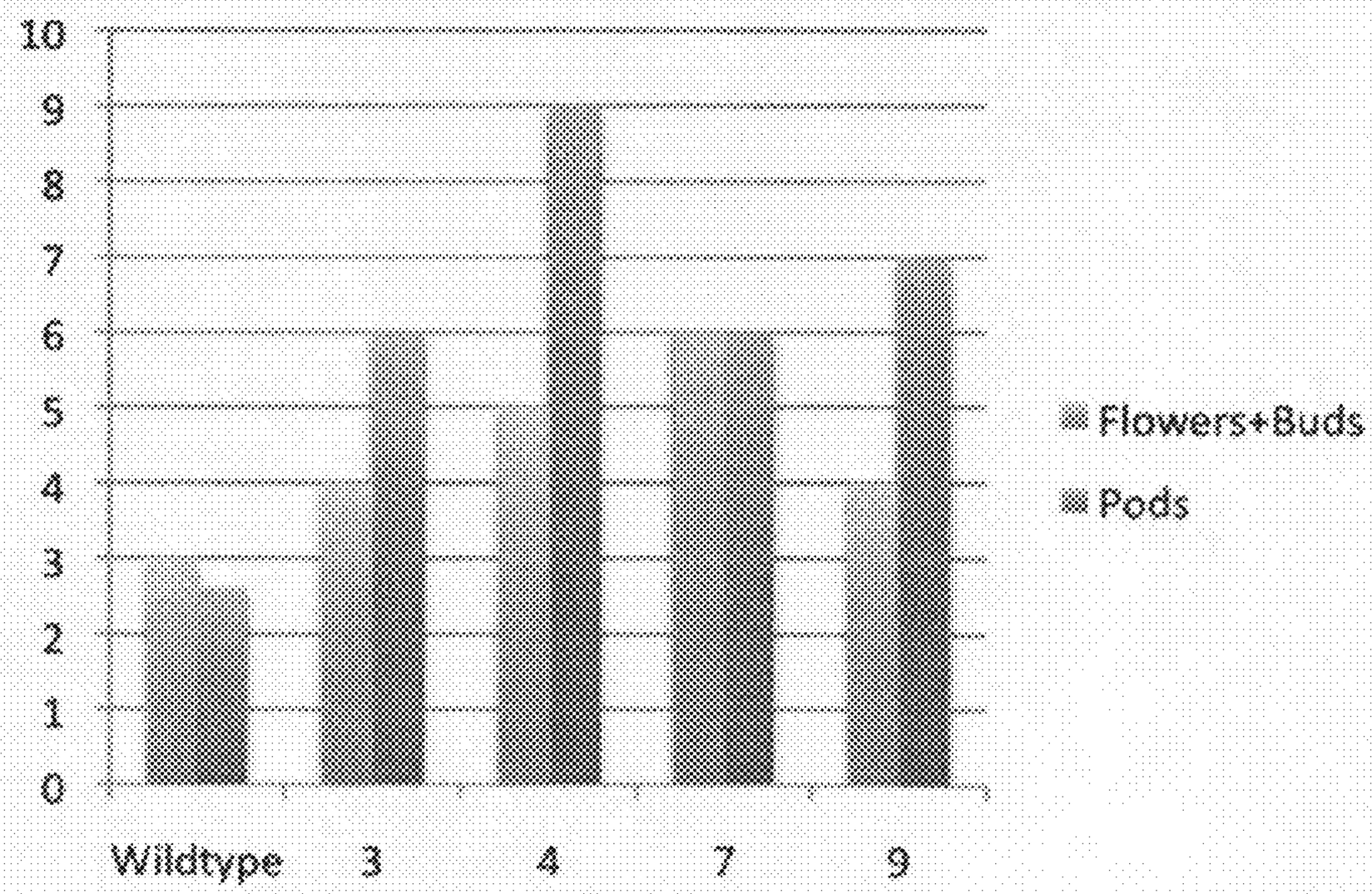

10
9
8
7
6
5
4
3
2
1
0
Wildtype
3
4
7
9
Flowers+Buds
Pods

FIG. 14C

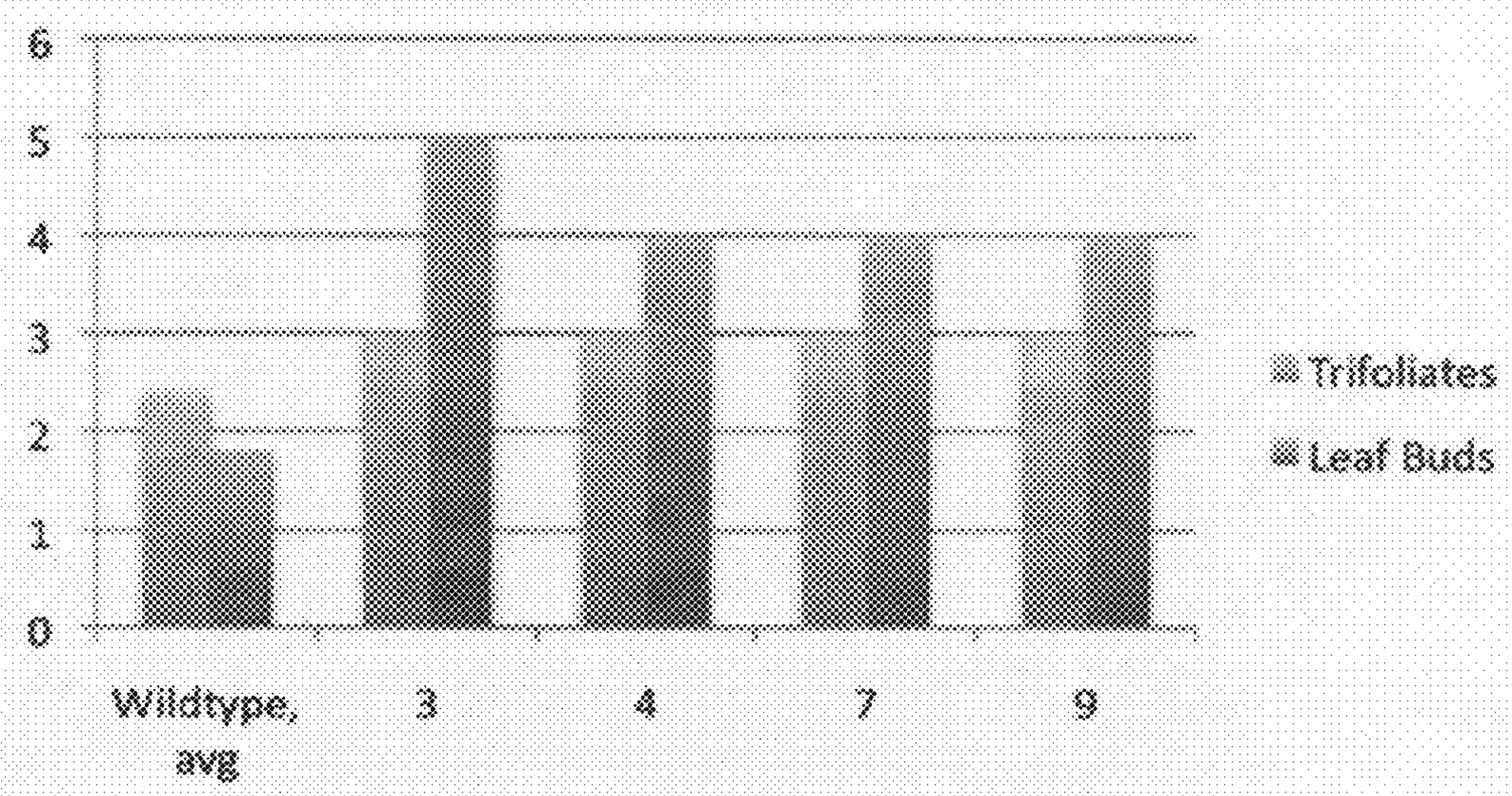
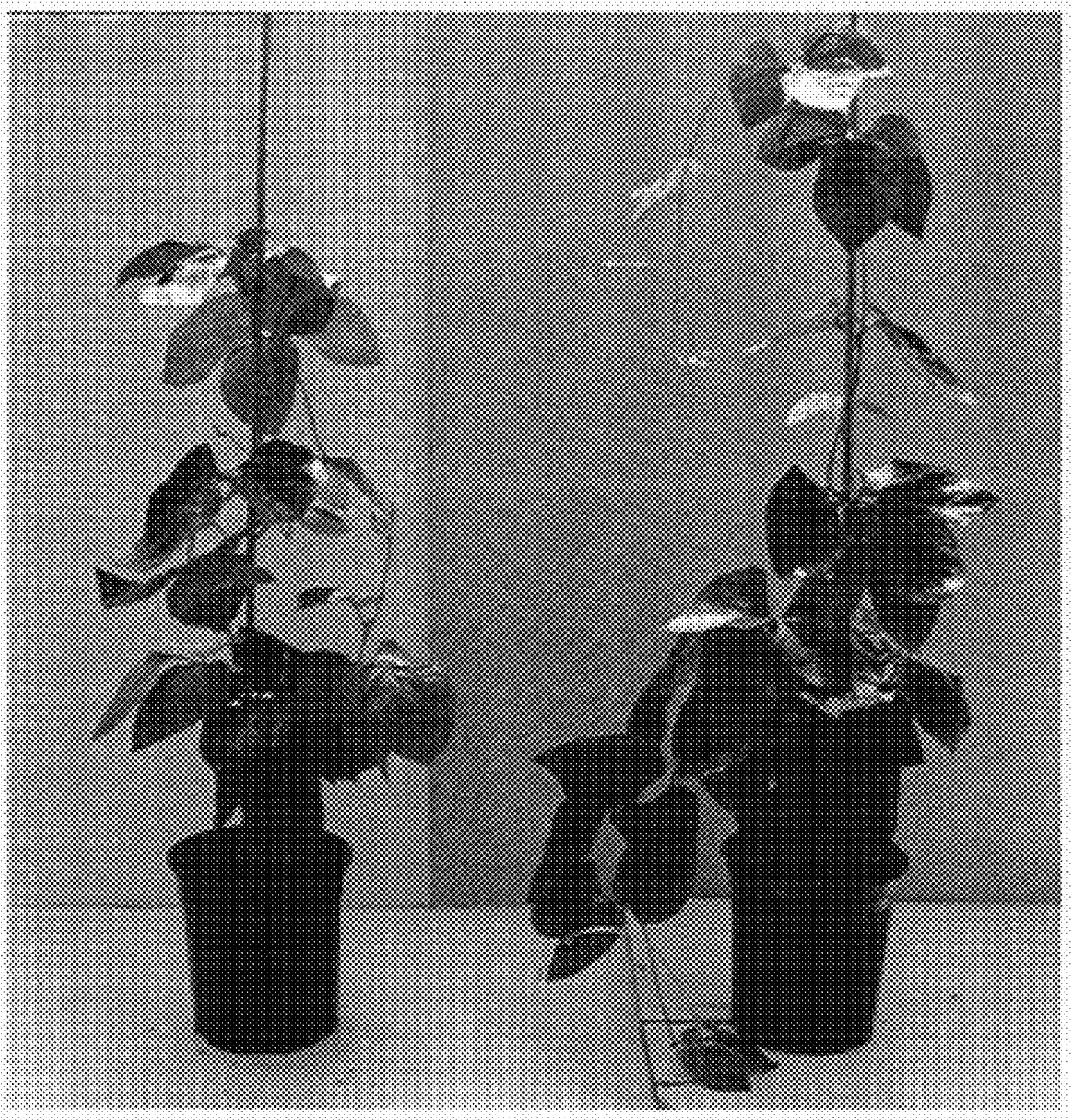


FIG. 15



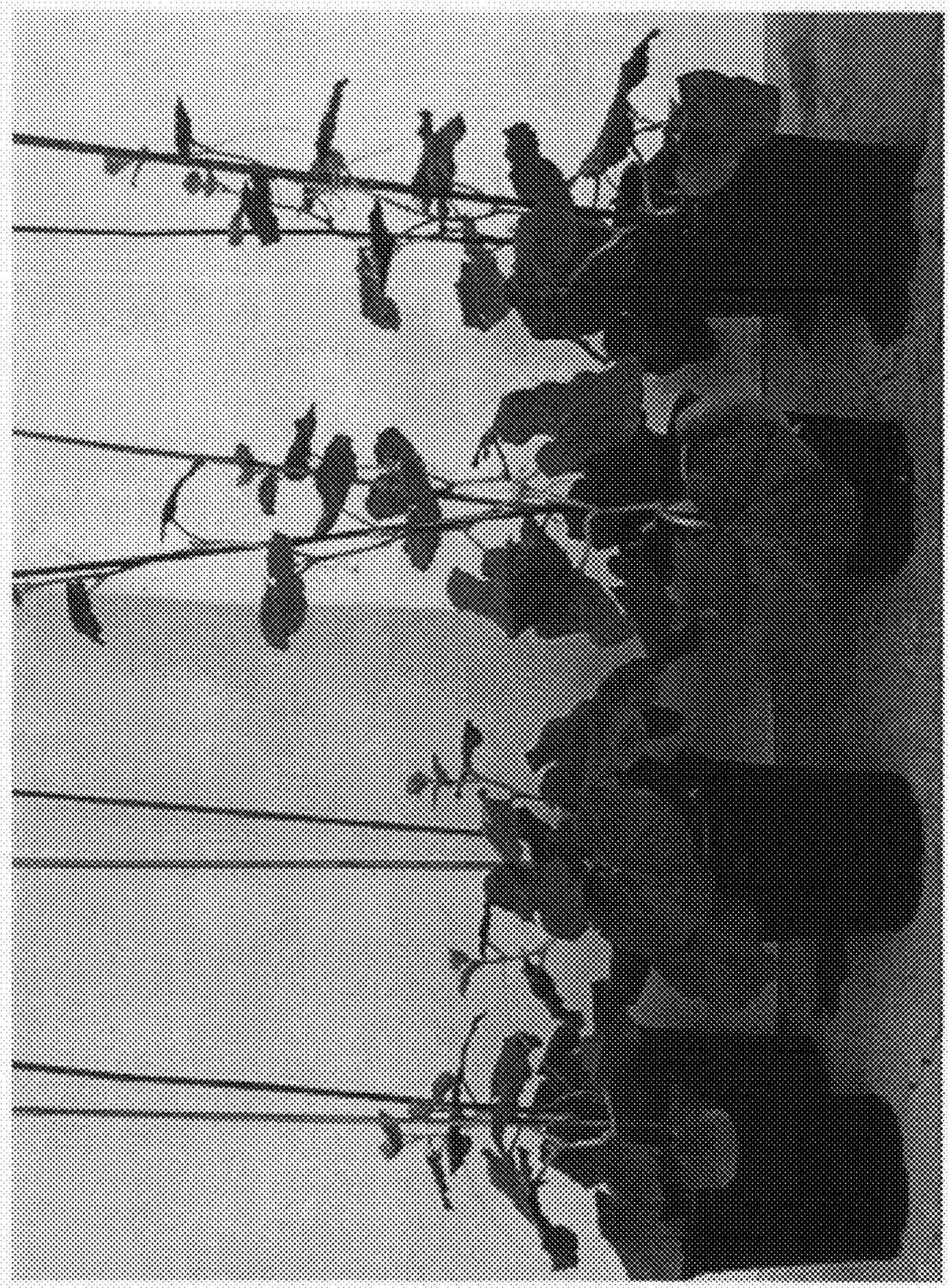


FIG. 16

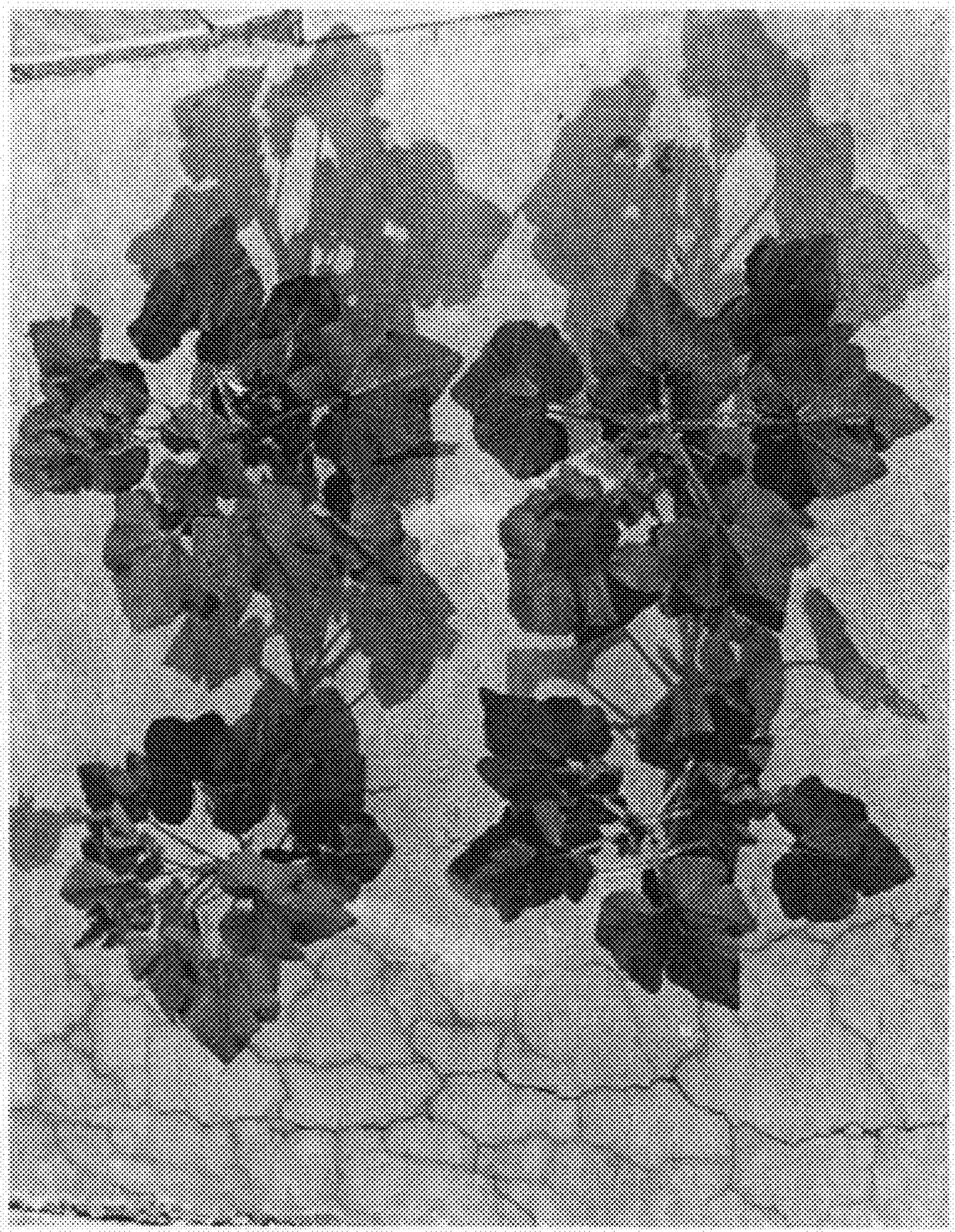


FIG. 17

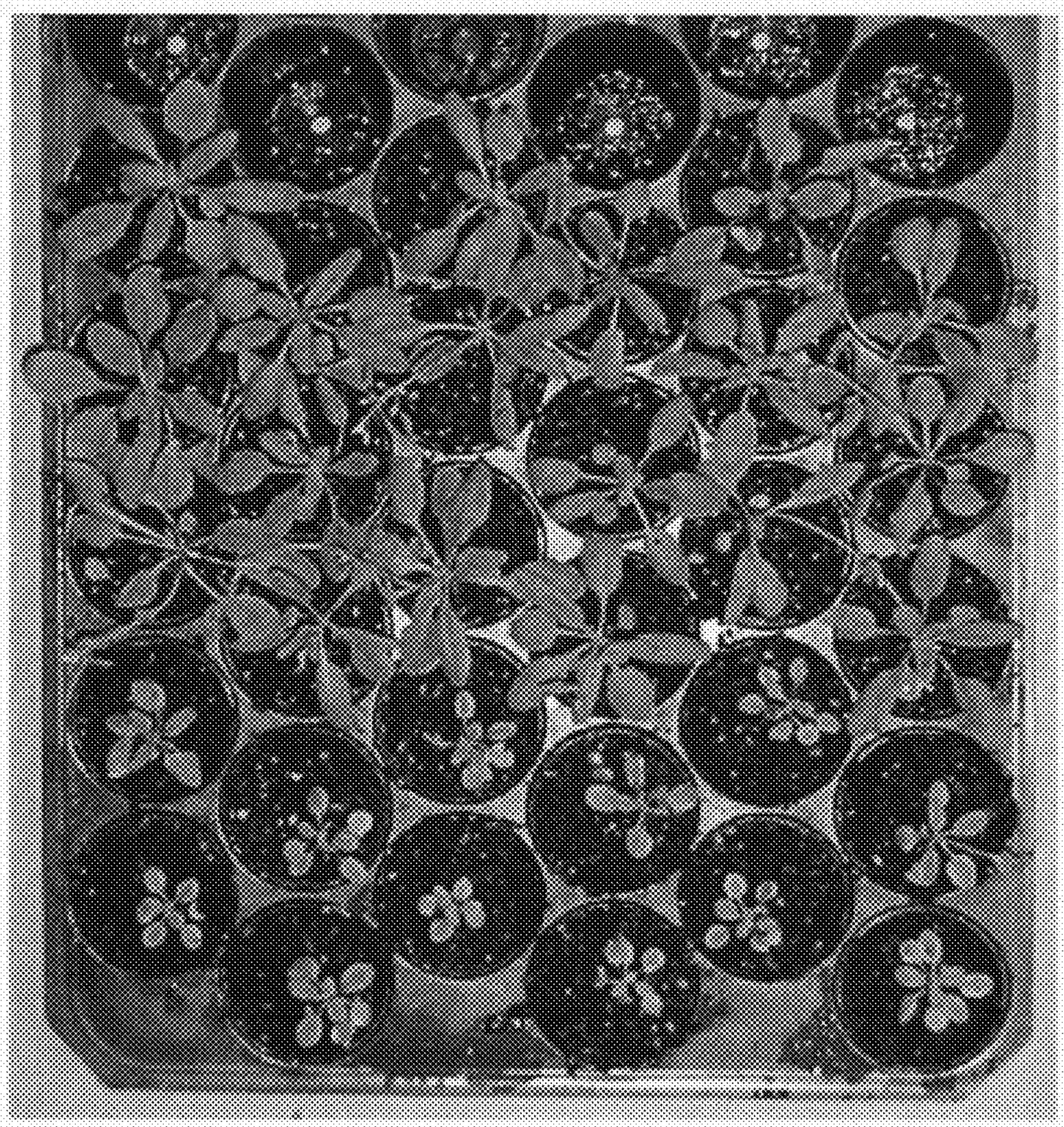
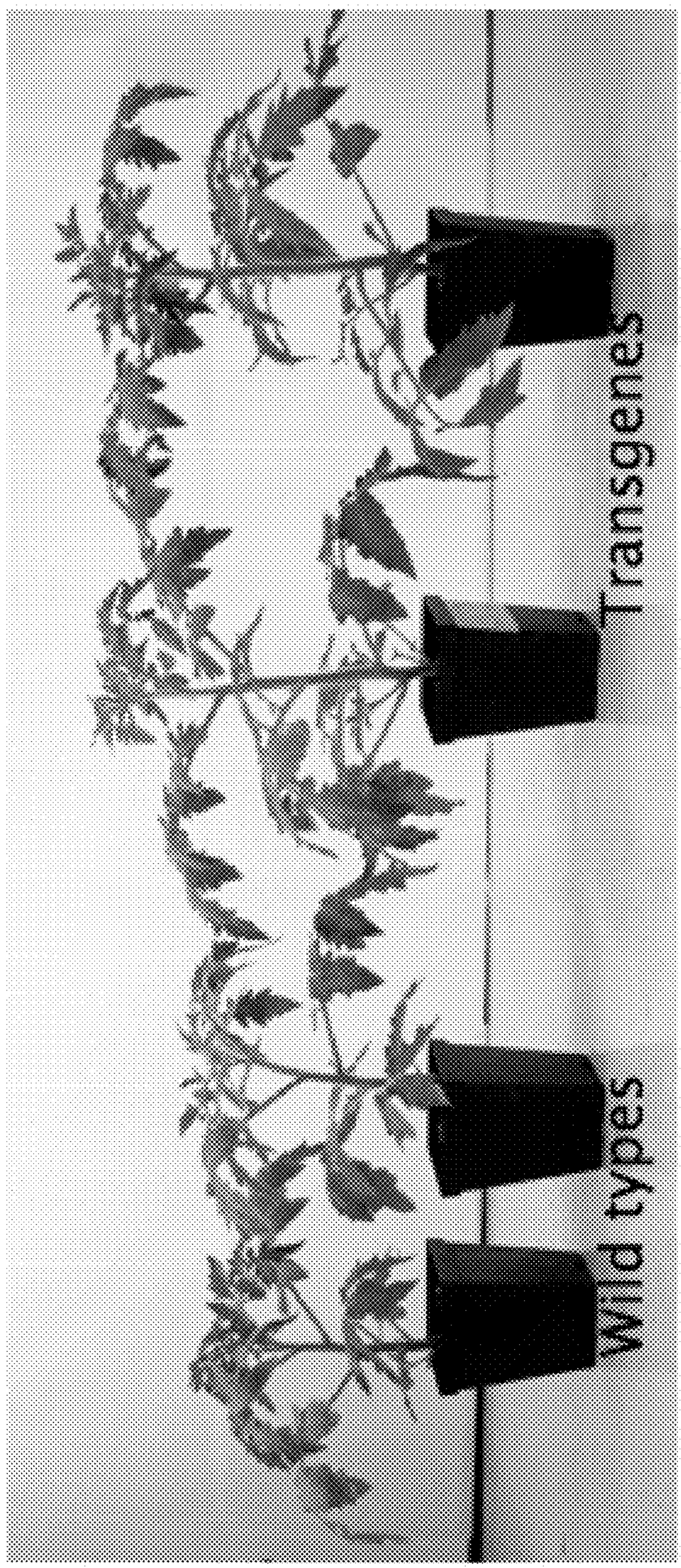


FIG. 18

FIG. 19A

FIG. 19B



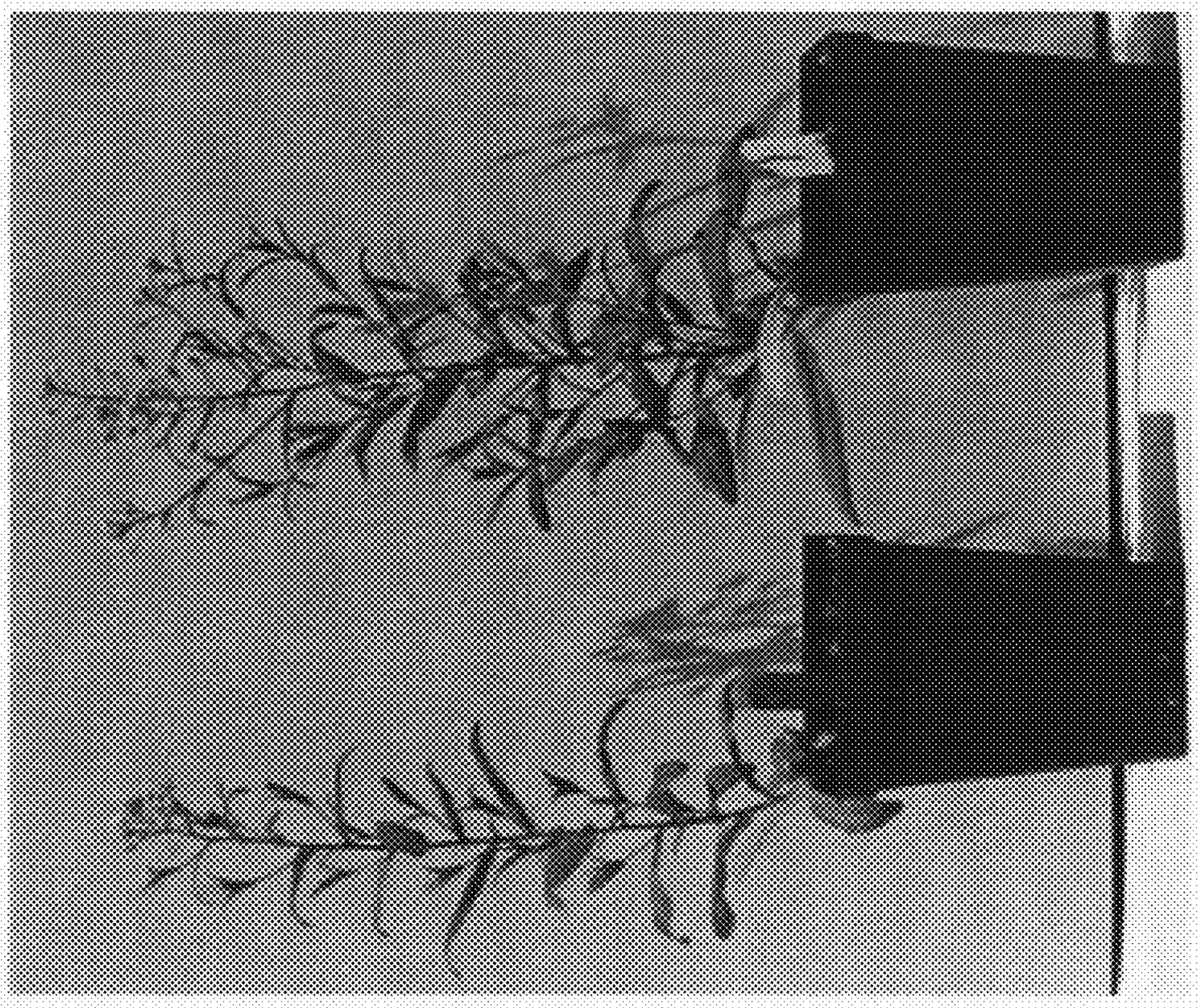


FIG. 20

TRANSGENIC PLANTS WITH ENHANCED GROWTH CHARACTERISTICS

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 12/551,271, filed Aug. 31, 2009, which application claims priority to U.S. Provisional Application No. 61/190,520 filed Aug. 29, 2008.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Contract No. W-7405-ENG-36 awarded by the United States Department of Energy to The Regents of The University of California, and Contract No. DE-AC52-06NA25396, awarded by the United States Department of Energy to Los Alamos National Security, LLC. The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] As the human population increases worldwide, and available farmland continues to be destroyed or otherwise compromised, the need for more effective and sustainable agriculture systems is of paramount interest to the human race. Improving crop yields, protein content, and plant growth rates represent major objectives in the development of agriculture systems that can more effectively respond to the challenges presented.

[0004] In recent years, the importance of improved crop production technologies has only increased as yields for many well-developed crops have tended to plateau. Many agricultural activities are time sensitive, with costs and returns being dependent upon rapid turnover of crops or upon time to market. Therefore, rapid plant growth is an economically important goal for many agricultural businesses that involve high-value crops such as grains, vegetables, berries and other fruits.

[0005] Genetic engineering has and continues to play an increasingly important yet controversial role in the development of sustainable agriculture technologies. A large number of genetically modified plants and related technologies have been developed in recent years, many of which are in widespread use today (*Factsheet: Genetically Modified Crops in the United States*, Pew Initiative on Food and Biotechnology, August 2004, http://pewagbiotech.org/resources/factsheets). The adoption of transgenic plant varieties is now very substantial and is on the rise, with approximately 250 million acres planted with transgenic plants in 2006.

[0006] While acceptance of transgenic plant technologies may be gradually increasing, particularly in the United States, Canada and Australia, many regions of the World remain slow to adopt genetically modified plants in agriculture, notably Europe. Therefore, consonant with pursuing the objectives of responsible and sustainable agriculture, there is a strong interest in the development of genetically engineered plants that do not introduce toxins or other potentially problematic substances into plants and/or the environment. There is also a strong interest in minimizing the cost of achieving objectives such as improving herbicide tolerance, pest and disease resistance, and overall crop yields. Accordingly, there remains a need for transgenic plants that can meet these objectives.

[0007] The goal of rapid plant growth has been pursued through numerous studies of various plant regulatory systems, many of which remain incompletely understood. In particular, the plant regulatory mechanisms that coordinate carbon and nitrogen metabolism are not fully elucidated. These regulatory mechanisms are presumed to have a fundamental impact on plant growth and development.

[0008] The metabolism of carbon and nitrogen in photosynthetic organisms must be regulated in a coordinated manner to assure efficient use of plant resources and energy. Current understanding of carbon and nitrogen metabolism includes details of certain steps and metabolic pathways which are subsystems of larger systems. In photosynthetic organisms, carbon metabolism begins with CO_2 fixation, which proceeds via two major processes, termed C-3 and C-4 metabolism. In plants with C-3 metabolism, the enzyme ribulose bisphosphate carboxylase (RuBisCo) catalyzes the combination of CO_2 with ribulose bisphosphate to produce 3-phosphoglycerate, a three carbon compound (C-3) that the plant uses to synthesize carbon-containing compounds. In plants with C-4 metabolism, CO_2 is combined with phosphoenol pyruvate to form acids containing four carbons (C-4), in a reaction catalyzed by the enzyme phosphoenol pyruvate carboxylase. The acids are transferred to bundle sheath cells, where they are decarboxylated to release CO_2, which is then combined with ribulose bisphosphate in the same reaction employed by C-3 plants.

[0009] Numerous studies have found that various metabolites are important in plant regulation of nitrogen metabolism. These compounds include the organic acid malate and the amino acids glutamate and glutamine. Nitrogen is assimilated by photosynthetic organisms via the action of the enzyme glutamine synthetase (GS) which catalyzes the combination of ammonia with glutamate to form glutamine. GS plays a key role in the assimilation of nitrogen in plants by catalyzing the addition of ammonium to glutamate to form glutamine in an ATP-dependent reaction (Miflin and Habash, 2002, Journal of Experimental Botany, Vol. 53, No. 370, pp. 979-987). GS also reassimilates ammonia released as a result of photorespiration and the breakdown of proteins and nitrogen transport compounds. GS enzymes may be divided into two general classes, one representing the cytoplasmic form (GS1) and the other representing the plastidic (i.e., chloroplastic) form (GS2).

[0010] Previous work has demonstrated that increased expression levels of GS1 result in increased levels of GS activity and plant growth, although reports are inconsistent. For example, Fuentes et al. reported that CaMV S35 promoter-driven overexpression of Alfalfa GS1 (cytoplasmic form) in tobacco resulted in increased levels of GS expression and GS activity in leaf tissue, increased growth under nitrogen starvation, but no effect on growth under optimal nitrogen fertilization conditions (Fuentes et al., 2001, J. Exp. Botany 52: 1071-81). Temple et al. reported that transgenic tobacco plants overexpressing the full length Alfalfa GS1 coding sequence contained greatly elevated levels of GS transcript, and GS polypeptide which assembled into active enzyme, but did not report phenotypic effects on growth (Temple et al., 1993, Molecular and General Genetics 236: 315-325). Corruzi et al. have reported that transgenic tobacco overexpressing a pea cytosolic GS1 transgene under the control of the CaMV S35 promoter show increased GS activity, increased cytosolic GS protein, and improved growth characteristics (U.S. Pat. No. 6,107,547). Unkefer et al. have more recently

reported that transgenic tobacco plants overexpressing the Alfalfa GS1 in foliar tissues, which had been screened for increased leaf-to-root GS activity following genetic segregation by selfing to achieve increased GS1 transgene copy number, were found to produce increased 2-hydroxy-5-oxoproline levels in their foliar portions, which was found to lead to markedly increased growth rates over wildtype tobacco plants (see, U.S. Pat. Nos. 6,555,500; 6,593,275; and 6,831,040).

[0011] Unkefer et al. have further described the use of 2-hydroxy-5-oxoproline (also known as 2-oxoglutaramate) to improve plant growth (U.S. Pat. Nos. 6,555,500; 6,593,275; 6,831,040). In particular, Unkefer et al. disclose that increased concentrations of 2-hydroxy-5-oxoproline in foliar tissues (relative to root tissues) triggers a cascade of events that result in increased plant growth characteristics. Unkefer et al. describe methods by which the foliar concentration of 2-hydroxy-5-oxoproline may be increased in order to trigger increased plant growth characteristics, specifically, by applying a solution of 2-hydroxy-5-oxoproline directly to the foliar portions of the plant and over-expressing glutamine synthetase preferentially in leaf tissues.

[0012] A number of transaminase and hydrolyase enzymes known to be involved in the synthesis of 2-hydroxy-5-oxoproline in animals have been identified in animal liver and kidney tissues (Cooper and Meister, 1977, CRC Critical Reviews in Biochemistry, pages 281-303; Meister, 1952, J. Biochem. 197: 304). In plants, the biochemical synthesis of 2-hydroxy-5-oxoproline has been known but has been poorly characterized. Moreover, the function of 2-hydroxy-5-oxoproline in plants and the significance of its pool size (tissue concentration) are unknown. Finally, the art provides no specific guidance as to precisely what transaminase(s) or hydrolase(s) may exist and/or be active in catalyzing the synthesis of 2-hydroxy-5-oxoproline in plants, and no such plant transaminases have been reported, isolated or characterized.

SUMMARY OF THE INVENTION

[0013] The invention relates to transgenic plants exhibiting dramatically enhanced growth rates, greater seed and fruit/pod yields, earlier and more productive flowering, more efficient nitrogen utilization, increased tolerance to high salt conditions, and increased biomass yields. In one embodiment, transgenic plants engineered to over-express both glutamine phenylpyruvate transaminase (GPT) and glutamine synthetase (GS) are provided. The GPT+GS double-transgenic plants of the invention consistently exhibit enhanced growth characteristics, with T0 generation lines showing an increase in biomass over wild type counterparts of between 50% and 300%. Generations that result from sexual crosses and/or selfing typically Perform even better, with some of the double-transgenic plants achieving an astounding four-fold biomass increase over wild type plants. Similarly, flower and fruit or pod yields are also tremendously improved, with T0 generation lines typically showing 50% to 70% increases over their wild type counterparts, and in some cases showing a 100% increase. Transgenic plants exhibiting such enhanced growth phenotypic characteristics have been successfully generated across a spectrum of individual plant species, using various transformation methodologies, different expression vectors and promoters, and heterologous and homologous transgene sequences from a variety of species, as exemplified by the numerous working examples provided herein. This invention, therefore, provides a fundamental break-though technology that has the potential to transform virtually all areas of agriculture.

[0014] Applicants have identified the enzyme glutamine phenylpyruvate transaminase (GPT) as a catalyst of 2-hydroxy-5-oxoproline (2-oxoglutaramate) synthesis in plants. 2-oxoglutaramate is a powerful signal metabolite which regulates the function of a large number of genes involved in the photosynthesis apparatus, carbon fixation and nitrogen metabolism. The invention provides isolated nucleic acid molecules encoding GPT, and discloses the novel finding that the encoded enzyme is directly involved in the synthesis of 2-hydroxy-5-oxoproline. This aspect of the invention is exemplified herein by the disclosure of GPT polynucleotides encoding GPTs from several species, including *Arabidopsis*, Grape, Rice, Soybean, Barley, Bamboo and a non-plant homolog from Zebra fish, most of which have been expressed as recombinant GPTs and confirmed as having GPT activity.

[0015] The invention further provides transgenic plants which express both a GPT transgene and a GS transgene. The expression of these two transgenes in such "double-transgene" plants results in a substantially increased rate of carbon dioxide fixation and an extremely potent growth enhancing effect, as these plants exhibit very significantly and sometimes tremendously enhanced growth rates and flower/fruit/pod/seed yields. Methods for the generation of such growth-enhanced transgenic plants are provided.

[0016] By preferentially increasing the concentration of the signal metabolite 2-oxoglutaramate (i.e., in foliar tissues), the transgenic plants of the invention are capable of producing higher overall yields over shorter periods of time, and therefore may provide agricultural industries with enhanced productivity across a wide range of crops. Importantly, unlike many transgenic plants described to date, the invention utilizes natural plant genes encoding a natural plant enzyme. The enhanced growth characteristics of the transgenic plants of the invention is achieved essentially by introducing additional GPT and GS capacity into the plant. Thus, the transgenic plants of the invention do not express any toxic substances, growth hormones, viral or bacterial gene products, and are therefore free of many of the concerns that have heretofore impeded the adoption of transgenic plants in certain parts of the World.

[0017] In one embodiment, the invention provides a transgenic plant comprising a GPT transgene and a GS transgene, wherein said GPT transgene and said GS transgene are operably linked to a plant promoter. In a specific embodiment, the GS transgene is a GS1 transgene. In another specific embodiment, the GPT transgene encodes a polypeptide having an amino acid sequence selected from the group consisting of (a) SEQ ID NO: 2; SEQ ID NO: 9; SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO 24, SEQ ID NO: 30, SEQ ID NO:31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 46 and SEQ ID NO: 49, and (b) an amino acid sequence that is at least 75% identical to any one of SEQ ID NO: 2; SEQ ID NO: 9; SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO 24, SEQ ID NO: 30, SEQ ID NO:31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 46 and SEQ ID NO: 49 and has GPT activity. In yet another specific embodiment, the GS transgene encodes a polypeptide having an amino acid sequence selected from the group consisting of (a) SEQ ID NO: 4, SEQ ID NO: 7 from residue 11 and SEQ ID NO: 41, and (b) an amino acid sequence that is at least 75% identical to SEQ ID NO: 4, SEQ

ID NO: 7 or SEQ ID NO: 41. In some embodiments, the GPT and GS transgenes are incorporated into the genome of the plant.

[0018] In a particular aspect of the invention, the GPT transgene and a GS transgene construct is incorporated into the genome of a plant selected from the group consisting of: maize, rice, sugar cane, wheat, oats, sorghum, switch grass, soya bean, tubers (such as potatoes), canola, lupins or cotton.

[0019] The invention also provides progeny of any generation of the transgenic plants of the invention, wherein said progeny comprises a GPT transgene and a GS transgene, as well as a seed of any generation of the transgenic plants of the invention, wherein said seed comprises said GPT transgene and said GS transgene. The transgenic plants of the invention may display one or more enhanced growth characteristics rate when compared to an analogous wild-type or untransformed plant, including without limitation increased growth rate, biomass yield, seed yield, flower or flower bud yield, fruit or pod yield, larger leaves, and may also display increased levels of GPT and/or GS activity, and/or increased levels of 2-oxoglutaramate. In some embodiments, the transgenic plants of the invention display increased nitrogen use efficiency or increased tolerance to salt or saline conditions.

[0020] In a further aspect of the invention there is provided a transplastomic plant or cell line carrying a GPT transgene and a GS transgene expression cassette, said expression cassette being flanked by sequences from the plant or plant cell's plastome.

[0021] Further still, the invention provides a method for preparing a transplastomic plant or cell line carrying a GPT transgene and a GS transgene construct, said method comprising the steps of: (a) inserting into at least one expression cassette at least a GPT transgene and a GS transgene, wherein said expression cassette is flanked by sequences from the plant or plant cell's plastome.

[0022] Methods for producing the transgenic plants of the invention and seeds thereof are also provided, including methods for producing a plant having enhanced growth properties, increased nitrogen use efficiency and increased tolerance to germination or growth in salt or saline conditions, relative to an analogous wild type or untransformed plant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0024] FIG. **1**. Nitrogen assimilation and 2-oxoglutaramate biosynthesis: schematic of metabolic pathway.

[0025] FIG. **2**. Photograph showing comparison of transgenic tobacco plants over-expressing either GS1 or GPT, compared to wild type tobacco plant. From left to right: wild type plant, Alfalfa GS1 transgene, *Arabidopsis* GPT transgene. See Examples 3 and 5, infra.

[0026] FIG. **3**. Photograph showing comparison of transgenic Micro-Tom tomato plants over-expressing either GS1 or GPT, compared to wild type tomato plant. From left to right: wild type plant, Alfalfa GS1 transgene, *Arabidopsis* GPT transgene. See Examples 4 and 6, infra.

[0027] FIG. **4**. Photographs showing comparisons of leaf sizes between wild type and GS1 or GPT transgenic tobacco plants. A: Comparison between leaves from GS1 transgenic tobacco (bottom leaf) and wild type (top leaf). B: Comparison between leaves from GPT transgenic tobacco (bottom leaf) and wild type (top leaf).

[0028] FIG. **5**. Photographs showing comparisons of transgenic tobacco plants generated from various crosses between GS1 and GPT transgenic tobacco lines with wild type and single transgene plants. A-C: Cross 2, 3 and 7, respectively. See Example 7, infra.

[0029] FIG. **6**. Photographs showing comparisons of leaf sizes between wild type and crosses between GS1 and GPT transgenic tobacco plants. A: Comparison between leaves from GSXGPT Cross 3 (bottom leaf) and wild type (top leaf). B: Comparison between leaves from GSXGPT Cross 7 (bottom leaf) and wild type (top leaf). See Example 7, infra.

[0030] FIG. **7**. Photograph of transgenic pepper plant (right) and wild type control pepper plant (left), showing larger pepper fruit yield in the transgenic plant relative to the wild type control plant. See Example 8, infra.

[0031] FIG. **8**. Transgenic bean plants compared to wild type control bean plants (several transgenic lines expressing *Arabidopsis* GPT and GS transgenes). Upper Left: plant heights on various days; Upper right: flower bud numbers; Lower left: flower numbers; Lower right: bean pod numbers. Wildtype is the control, and lines 2A, 4A and 5B are all transgenic plant lines. See Example 9, infra.

[0032] FIG. **9**. Photograph of transgenic bean plant (right) and wild type control bean plant (left), showing increased growth in the transgenic plant relative to the wild type control plant. Transgenic line expressing *Arabidopsis* GPT and GS transgenes. See Example 9, infra.

[0033] FIG. **10**. Transgenic bean plants pods, flowers and flower buds compared to wild type control bean plants (transgenic line expressing grape GPT and *Arabidopsis* GS transgenes). See Example 10, infra.

[0034] FIG. **11**. Photograph of transgenic bean plant (right) and wild type control bean plant (left), showing increased growth in the transgenic plant relative to the wild type control plant. Transgenic line expressing Grape GPT and *Arabidopsis* GS transgenes. See Example 10, infra.

[0035] FIG. **12**. Transgenic Cowpea Line A plants compared to wild type control Cowpea plants (transgenic line expressing *Arabidopsis* GPT and GS transgenes), showing that the transgenic plants grow faster and flower and set pods sooner than wild type control plants. (A) Relative height and longest leaf measurements as of May 21, (B) Relative trifolate leafs and flower buds as of June 18, (C) Relative numbers of flowers, flower buds and pea pods as of June 22. See Example 11, infra.

[0036] FIG. **13**. Photograph of transgenic Cowpea Line A plant (right) and wild type control Cowpea plant (left), showing increased growth in the transgenic plant relative to the wild type control plant. Transgenic line expressing *Arabidopsis* GPT and GS transgenes. See Example 11, infra.

[0037] FIG. **14**. Transgenic Cowpea Line G plants compared to wild type control Cowpea plants (transgenic line expressing Grape GPT and *Arabidopsis* GS transgenes), showing that the transgenic plants grow faster and flower and set pods sooner than wild type control plants. (A) plant heights, (B) flowers and pea pod numbers, (C) leaf bud and trifolate numbers. See Example 12, infra.

[0038] FIG. **15**. Photograph of transgenic Cowpea Line G plant (right) and wild type control Cowpea plant (left), showing increased growth in the transgenic plant relative to the

wild type control plant. Transgenic line expressing Grape GPT and *Arabidopsis* GS transgenes. See Example 12, infra.

[0039] FIG. **16**. Photograph of transgenic Cantaloupe plant (right) and wild type control Cantaloupe plant (left), showing increased growth in the transgenic plant relative to the wild type control plant. Transgenic line expressing *Arabidopsis* GPT and GS transgenes. See Example 14, infra.

[0040] FIG. **17**. Photograph of transgenic Pumpkin plants (right) and wild type control Pumpkin plants (left), showing increased growth in the transgenic plants relative to the wild type control plants. Transgenic lines expressing *Arabidopsis* GPT and GS transgenes. See Example 15, infra.

[0041] FIG. **18**. Photograph of transgenic *Arabidopsis* plants (right) and wild type control *Arabidopsis* plants (left), showing increased growth in the transgenic plants relative to the wild type control plants. Transgenic lines expressing *Arabidopsis* GPT and GS transgenes. See Example 16, infra.

[0042] FIG. **19**. Transgenic tomato plants expressing *Arabidopsis* GPT and GS transgenes compared to control tomato plants. (A) Photograph of transgenic tomato plant leaves (right) vs. wild type control leaves (left) showing larger leaves in the transgenic plant. (B) Photograph of transgenic tomato plants (right) and wild type control plants (left), showing increased growth in the transgenic plants relative to the wild type control plants. See Example 17, infra.

[0043] FIG. **20**. Photograph of transgenic Camelina plant (right) and wild type control Camelina plant (left), showing increased growth in the transgenic plant relative to the wild type control plant. Transgenic line expressing *Arabidopsis* GPT and GS transgenes. See Example 18, infra.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0044] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (Ausbel et al., eds., John Wiley & Sons, Inc. 2001; Transgenic Plants: Methods and Protocols (Leandro Pena, ed., Humana Press, 1st edition, 2004); and, *Agrobacterium* Protocols (Wan, ed., Humana Press, 2nd edition, 2006). As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

[0045] Each document, reference, patent application or patent cited in this text is expressly incorporated herein in its entirety by reference, and each should be read and considered as part of this specification. That the document, reference, patent application or patent cited in this specification is not repeated herein is merely for conciseness.

[0046] The term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof ("polynucleotides") in either single- or double-stranded form. Unless specifically limited, the term "polynucleotide" encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., 1991, Nucleic Acid Res. 19: 5081; Ohtsuka et al., 1985 J. Biol. Chem. 260: 2605-2608; and Cassol et al., 1992; Rossolini et al., 1994, Mol. Cell. Probes 8: 91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0047] The term "promoter" refers to anucleic acid control sequence or sequences that direct transcription of an operably linked nucleic acid. As used herein, a "plant promoter" is a promoter that functions in plants. Promoters include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0048] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0049] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure

that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0050] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0051] The term "plant" includes whole plants, plant organs (e.g., leaves, stems, flowers, roots, reproductive organs, embryos and parts thereof, etc.), seedlings, seeds and plant cells and progeny thereof. The class of plants which can be used in the method of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), as well as gymnosperms. It includes plants of a variety of ploidy levels, including polyploid, diploid, haploid and hemizygous.

[0052] The terms "GPT polynucleotide" and "GPT nucleic acid" are used interchangeably herein, and refer to a full length or partial length polynucleotide sequence of a gene which encodes a polypeptide involved in catalyzing the synthesis of 2-oxoglutaramate, and includes polynucleotides containing both translated (coding) and un-translated sequences, as well as the complements thereof. The term "GPT coding sequence" refers to the part of the gene which is transcribed and encodes a GPT protein. The term "targeting sequence" refers to the amino terminal part of a protein which directs the protein into a subcellular compartment of a cell, such as a chloroplast in a plant cell. GPT polynucleotides are further defined by their ability to hybridize under defined conditions to the GPT polynucleotides specifically disclosed herein, or to PCR products derived therefrom.

[0053] A "GPT transgene" is a nucleic acid molecule comprising a GPT polynucleotide which is exogenous to transgenic plant, or plant embryo, organ or seed, harboring the nucleic acid molecule, or which is exogenous to an ancestor plant, or plant embryo, organ or seed thereof, of a transgenic plant harboring the GPT polynucleotide. More particularly, the exogenous GPT transgene will be heterogeneous with any GPT polynucleotide sequence present in wild-type plant, or plant embryo, organ or seed into which the GPT transgene is inserted. To this extent the scope of the heterogeneity required need only be a single nucleotide difference. However, preferably the heterogeneity will be in the order of an identity between sequences selected from the following identities: 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, and 20%.

[0054] The terms "GS polynucleotide" and "GS nucleic acid" are used interchangeably herein, and refer to a full length or partial length polynucleotide sequence of a gene which encodes a glutamine synthetase protein, and includes polynucleotides containing both translated (coding) and un-translated sequences, as well as the complements thereof. The term "GS coding sequence" refers to the part of the gene which is transcribed and encodes a GS protein. The terms "GS1 polynucleotide" and "GS1 nucleic acid" are used interchangeably herein, and refer to a full length or partial length polynucleotide sequence of a gene which encodes a glutamine synthetase isoform 1 protein, and includes polynucleotides containing both translated (coding) and un-translated sequences, as well as the complements thereof. The term "GS1 coding sequence" refers to the part of the gene which is transcribed and encodes a GS1 protein.

[0055] A "GS transgene" is a nucleic acid molecule comprising a GS polynucleotide which is exogenous to transgenic plant, or plant embryo, organ or seed, harboring the nucleic acid molecule, or which is exogenous to an ancestor plant, or plant embryo, organ or seed thereof, of a transgenic plant harboring the GS polynucleotide. A "GS1 transgene" is a nucleic acid molecule comprising a GS1 polynucleotide which is exogenous to transgenic plant, or plant embryo, organ or seed, harboring the nucleic acid molecule, or which is exogenous to an ancestor plant, or plant embryo, organ or seed thereof, of a transgenic plant harboring the GS1 polynucleotide. More particularly, the exogenous GS or GS1 transgene will be heterogeneous with any GS or GS1 polynucleotide sequence present in wild-type plant, or plant embryo, organ or seed into which the GS or GS1 transgene is inserted. To this extent the scope of the heterogeneity required need only be a single nucleotide difference. However, preferably the heterogeneity will be in the order of an identity between sequences selected from the following identities: 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, 15%, and 20%.

[0056] Exemplary GPT polynucleotides of the invention are presented herein, and include GPT coding sequences for *Arabidopsis*, Rice, Barley, Bamboo, Soybean, Grape, Clementine orange and Zebra Fish GPTs.

[0057] Partial length GPT polynucleotides include polynucleotide sequences encoding N- or C-terminal truncations of GPT, mature GPT (without targeting sequence) as well as sequences encoding domains of GPT. Exemplary GPT polynucleotides encoding N-terminal truncations of GPT include *Arabidopsis*-30, -45 and -56 constructs, in which coding sequences for the first 30, 45, and 56, respectively, amino acids of the full length GPT structure of SEQ ID NO: 2 are eliminated.

[0058] In employing the GPT polynucleotides of the invention in the generation of transformed cells and transgenic plants, one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only "substantially identical" to a sequence of the gene from which it was derived, as further defined below. The term "GPT polynucleotide" specifically encompasses such substantially identical variants. Similarly, one of skill will recognize that because of codon degeneracy, a number of polynucleotide sequences will encode the same polypeptide, and all such polynucleotide sequences are meant to be included in the term GPT polynucleotide. In addition, the term specifically includes those sequences substantially identical (determined as described below) with an GPT polynucleotide sequence disclosed herein and that encode polypeptides that are either mutants of wild type GPT polypeptides or retain the function of the GPT polypeptide (e.g., resulting from conservative substitutions of amino acids in a GPT polypeptide). The term "GPT polynucleotide" therefore also includes such substantially identical variants.

[0059] The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified

by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0060] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0061] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), *Glycine* (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, *Proteins* (1984)).

[0062] Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, see, e.g., Alberts et al., *Molecular Biology of the Cell* (3rd ed., 1994) and Cantor and Schimmel, *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (1980). "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 25 to approximately 500 amino acids long. Typical domains are made up of sections of lesser organization such as stretches of β-sheet and α-helices. "Tertiary structure" refers to the complete three dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the non-covalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

[0063] The term "isolated" refers to material which is substantially or essentially free from components which normally accompany the material as it is found in its native or natural state. However, the term "isolated" is not intended refer to the components present in an electrophoretic gel or other separation medium. An isolated component is free from such separation media and in a form ready for use in another application or already in use in the new application/milieu. An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0064] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, a nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a nucleic acid encoding a protein from one source and a nucleic acid encoding a peptide sequence from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0065] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 70% identity, preferably 75%, 80%, 85%, 90%, or 95% identity over a specified region, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using a sequence comparison algorithms, or by manual alignment and visual inspection. This definition also refers to the complement of a test sequence, which has substantial sequence or subsequence complementarity when the test sequence has substantial identity to a reference sequence. This definition also refers to the complement of a test sequence, which has substantial sequence or subsequence complementarity when the test sequence has substantial identity to a reference sequence.

[0066] When percentage of sequence identity is used in reference to polypeptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the polypeptide. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution.

[0067] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0068] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, 1981, Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman & Wunsch, 1970, J. Mol. Biol. 48:443, by the search for similarity method of Pearson & Lipman, 1988, Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0069] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., 1977, Nuc. Acids Res. 25:3389-3402 and Altschul et al., 1990, J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 are used, typically with the default parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=−4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=−4, and a comparison of both strands.

[0070] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, 1993, Proc. Nat'l. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0071] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, highly stringent conditions are selected to be about 5-10° C. lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. Low stringency conditions are generally selected to be about 15-30° C. below the Tm. Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0M sodium ion, typically about 0.01 to 1.0M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization.

[0072] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cased, the nucleic acids typically hybridize under moderately stringent hybridization conditions.

[0073] Genomic DNA or cDNA comprising GPT polynucleotides may be identified in standard Southern blots under stringent conditions using the GPT polynucleotide sequences disclosed here. For this purpose, suitable stringent conditions for such hybridizations are those which include a hybridization in a buffer of 40% formamide, 1M NaCl, 1% SDS at 37° C., and at least one wash in 0.2×SSC at a temperature of at least about 50° C., usually about 55° C. to about 60° C., for 20 minutes, or equivalent conditions. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions may be utilized to provide conditions of similar stringency.

[0074] A further indication that two polynucleotides are substantially identical is if the reference sequence, amplified by a pair of oligonucleotide primers, can then be used as a probe under stringent hybridization conditions to isolate the

test sequence from a cDNA or genomic library, or to identify the test sequence in, e.g., a northern or Southern blot.

Transgenic Plants:

[0075] The invention provides novel transgenic plants exhibiting substantially enhanced agronomic characteristics, including faster growth, greater mature plant fresh weight and total biomass, earlier and more abundant flowering, and greater fruit, pod and seed yields. The transgenic plants of the invention are generated by introducing into a plant one or more expressible genetic constructs capable of driving the expression of one or more polynucleotides encoding glutamine synthetase (GS) and glutamine phenylpyruvate transaminase (GPT). In an exemplary embodiment, single-transgene parental lines carrying either a GPT or GS1 transgene coding sequence are generated, preferably selfed until homozygous for the transgene, then crossed to generate progeny plants containing both transgenes.

[0076] The transgenic plants of the invention may be any vascular plant of the phylum Tracheophyta, including angiosperms and gymnosperms. Angiosperms may be a monocotyledonous (monocot) or a dicotyledonous (dicot) plant. Important monocots include those of the grass families, such as the family Poaceae and Gramineae, including plants of the genus *Avena* (*Avena sativa*, oats), genus *Hordeum* (i.e., *Hordeum vulgare*, Barley), genus *Oryza* (i.e., *Oryza sativa*, rice, cultivated rice varieties), genus *Panicum* (*Panicum* spp., *Panicum virgatum*, Switchgrass), genus *Phleum* (*Phleum pratense*, Timothy-grass), genus *Saccharum* (i.e., *Saccharum officinarum, Saccharum spontaneum*, hybrids thereof, Sugarcane), genus *Secale* (i.e., *Secale cereale*, Rye), genus *Sorghum* (*Sorghum vulgare, Sorghum*), genus *Triticum* (wheat, various classes, including *T. aestivum* and *T. durum*), genus *Fagopyrum* (buckwheat, including *F. esculentum*), genus *Triticosecale* (Triticale, various hybrids of wheat and rye), genus *Chenopodium* (quinoa, including *C. quinoa*), genus *Zea* (i.e., *Zea mays*, numerous varieties) as well as millets (i.e., *Pennisetum glaucum*) including the genus *Digitaria* (*D. exilis*).

[0077] Important dicots include those of the family Solanaceae, such as plants of the genus *Lycopersicon* (*Lycopersicon esculentum*, tomato), genus *Capiscum* (*Capsicum annuum*, peppers), genus *Solanum* (*Solanum tuberosum*, potato, *S. lycopersicum*, tomato); genus *Manihot* (cassaya, *M. esculenta*), genus *Ipomoea* (sweet potato, *I. batatas*), genus *Olea* (olives, including *O. europaea*); plants of the *Gossypium* family (i.e., *Gossypium* spp., *G. hirsutum, G. herbaceum*, cotton); the Legumes (family Fabaceae), such as peas (*Pisum* spp, *P. sativum*), beans (*Glycine* spp., *Glycine max* (soybean); *Phaseolus vulgaris*, common beans, *Vigna radiata*, mung bean), chickpeas (*Cicer arietinum*)), lentils (*Lens culinaris*), peanuts (*Arachis hypogaea*); coconuts (*Cocos nucifera*) as well as various other important crops such as *camelina* (*Camelina sativa*, family Brassicaceae), citrus (*Citrus* spp, family Rutaceae), coffee (*Coffea* spp, family Rubiaceae), melon (*Cucumis* spp, family Cucurbitaceae), squash (*Cucurbita* spp, family Cucurbitaceae), roses (*Rosa* spp, family Rosaceae), sunflower (*Helianthus annuus*, family Asteraceae), sugar beets (*Beta* spp, family Amaranthaceae), including sugarbeet, *B. vulgaris*), genus *Daucus* (carrots, including *D. carota*), genus *Pastinaca* (parsnip, including *P. sativa*), genus *Raphanus* (radish, including *R. sativus*), genus *Dioscorea* (yams, including *D. rotundata* and *D. cayenensis*), genus *Armoracia* (horseradish, including *A. rusticana*), genus *Elaeis* (Oil palm, including *E. guineensis*), genus *Linum* (flax, including *L. usitatissimum*), genus *Carthamus* (safflower, including *C. tinctorius* L.), genus *Sesamum* (sesame, including *S. indicum*), genus *Vitis* (grape, including *Vitis vinifera*), and plants of the genus *Brassica* (family Brassicaceae, i.e., broccoli, brussel sprouts, cabbage, swede, turnip, rapeseed *B. napus*, and cauliflower).

[0078] Other specific plants which may be transformed to generate the transgenic plants of the invention include various other fruits and vegetables, such as apples, asparagus, avocado, banana, blackberry, blueberry, brussel sprout, cabbage, cotton, canola, carrots, radish, cucumbers, cherries, cranberries, cantaloupes, eggplant, grapefruit, lemons, limes, nectarines, oranges, peaches, pineapples, pears, plums, tangelos, tangerines, papaya, mango, strawberry, raspberry, lettuce, onion, grape, kiwi fruit, okra, parsnips, pumpkins, and spinach. In addition various flowering plants, trees and ornamental plants may be used to generate transgenic varietals, including without limitation lily, carnation, chrysanthemum, petunia, geranium, violet, gladioli, lupine, orchid and lilac.

[0079] In stable transformation embodiments of the invention, one or more copies of the expressible genetic construct become integrated into the host plant genome, thereby providing increased GS and GPT enzyme capacity into the plant, which serves to mediate increased synthesis of 2-oxoglutaramate, which in turn signals metabolic gene expression, resulting in increased plant growth and the enhancement other agronomic characteristics. 2-oxoglutaramate is a metabolite which is an extremely potent effector of gene expression, metabolism and plant growth (U.S. Pat. No. 6,555,500), and which may play a pivotal role in the coordination of the carbon and nitrogen metabolism systems (Lancien et al., 2000, *Enzyme Redundancy and the Importance of 2-Oxoglutarate in Higher Plants Ammonium Assimilation*, Plant Physiol. 123: 817-824). See, also, the schematic of the 2-oxoglutaramate pathway shown in FIG. **1**.

[0080] In one aspect of the invention, applicants have isolated a nucleic acid molecule encoding the *Arabidopsis* glutamine phenylpyruvate transaminase (GPT) enzyme (see Example 1, infra), and have demonstrated for the first time that the expressed recombinant enzyme is active and capable of catalyzing the synthesis of the signal metabolite, 2-oxoglutaramate (Example 2, infra). Further, applicants have demonstrated for the first time that over-expression of the *Arabidopsis* glutamine transaminase gene in a transformed heterologous plant results in enhanced CO_2 fixation rates and increased growth characteristics (Example 3, infra).

[0081] Applicants' previous work demonstrated that over-expression of Alfalfa GS1 gene under the control of a strong constitutive promoter results in transgenic tobacco plants with higher levels of GS activity in the leaves. These plants outgrow their wild-type counterparts, fix CO_2 faster, contain increased concentrations of total protein, as well as increased concentrations of glutamine and 2-oxoglutaramate, and show increased rates of uptake of nitrate through their roots.

[0082] As disclosed herein (see Example 3, infra), over-expression of a transgene comprising the full-length *Arabidopsis* GPT coding sequence in transgenic tobacco plants also results in faster CO_2 fixation, and increased levels of total protein, glutamine and 2-oxoglutaramate. These transgenic plants also grow faster than wild-type plants (FIG. **2**). Similarly, in preliminary studies conducted with tomato plants (see Example 4, infra), tomato plants transformed with the *Arabidopsis* GPT transgene showed significant enhancement

of growth rate, flowering, and seed yield in relation to wild type control plants (FIG. **3** and Example 4, infra).

[0083] In one particular embodiment, exemplified herein by way of Examples 3, 5 and 7, infra, a first set of parental single-transgene tobacco plant lines carrying the Alfalfa GS1 gene, including 5' and 3' untranslated regions, were generated using *Agrobacterium* mediated gene transformation, under selective pressure, together with screening for the fastest growing phenotype, and selfing to transgene/phenotype homozygosity (see Example 5, infra). A second set of parental single-transgene tobacco plant lines carrying the full length coding sequence of *Arabidopsis* GPT were generated in the same manner (Example 3, infra). High growth rate performing plants from each of the parental lines were then sexually crossed to yield progeny lines (Example 7, infra).

[0084] The resulting progeny from multiple crosses of *Arabidopsis* GS1 and GPT transgenic tobacco plants produce far better and quite surprising increases in growth rates over the single-transgene parental lines as well as wildtype plants. FIG. **5** shows photographs of double-transgene progeny from single-transgene GS1×GPT plant crosses, relative to wild type and single-transgene parental plants. FIG. **6** shows photographs comparing leaf sizes of double-transgene progeny and wild type plants. Experimentally observed growth rates in these double transgenic plants ranged between 200% and 300% over wild-type plants (Example 7, infra). Moreover, total biomass levels increased substantially in the double-transgene plants, with whole plant fresh weights typically being about two to three times the wild-type plant weights. Similarly, seed yields showed similar increases in the double-transgene plants, with seed pod production typically two to three times the wild type average, and overall seed yields exceeding wild-type plant yields by 300-400%.

[0085] In addition to the transgenic tobacco plants referenced above, various other species of transgenic plants comprising GPT and GS transgenes are specifically exemplified herein. As exemplified herein, transgenic plants showing enhanced growth characteristics have been generated in two species of Tomato (see Examples 4 and 17), Pepper (Example 8), Beans (Examples 9 and 10), Cowpea (Examples 11 and 12), Alfalfa (Example 13), Cantaloupe (Example 14), Pumpkin (Example 15), *Arabidopsis* (Example 16) and Camilena (Example 18). These transgenic plants of the invention were generated using a variety of transformation methodologies, including *Agrobacterium*-mediated callus, floral dip, seed inoculation, pod inoculation, and direct flower inoculation, as well as combinations thereof, and via sexual crosses of single transgene plants, as exemplified herein. Different GPT and GS transgenes were successfully employed in generating the transgenic plants of the invention, as exemplified herein.

[0086] The invention also provides methods of generating a transgenic plant having enhanced growth and other agronomic characteristics. In one embodiment, a method of generating a transgenic plant having enhanced growth and other agronomic characteristics comprises introducing into a plant cell an expression cassette comprising a nucleic acid molecule encoding a GPT transgene, under the control of a suitable promoter capable of driving the expression of the transgene, so as to yield a transformed plant cell, and obtaining a transgenic plant which expresses the encoded GPT. In another embodiment, a method of generating a transgenic plant having enhanced growth and other agronomic characteristics comprises introducing into a plant cell one or more nucleic acid constructs or expression cassettes comprising nucleic acid molecules encoding a GPT transgene and an GS transgene, under the control of one or more suitable promoters (and, optionally, other regulatory elements) capable of driving the expression of the transgenes, so as to yield a plant cell transformed thereby, and obtaining a transgenic plant which expresses the GPT and GS transgenes.

[0087] Based on the results disclosed herein, it is clear that any number of GPT and GS polynucleotides may be used to generate the transgenic plants of the invention. Both GS1 and GPT proteins are highly conserved among various plant species, and it is evident from the experimental data disclosed herein that closely-related non-plant GPTs may be used as well (e.g., *Danio rerio* GPT). With respect to GPT, numerous GPT polynucleotides derived from different species have been shown to be active and useful as GPT transgenes. Similarly, different GS polynucleotides may be used, including without limitation any plant GS1 encoding polynucleotide that generates GS activity in a host cell transformed with an expressible GS1 construct.

[0088] In a specific embodiment, the GPT transgene is a GPT polynucleotide encoding an *Arabidopsis* derived GPT, such as the GPT of SEQ ID NO: 2, SEQ ID NO: 21 and SEQ ID NO: 30, and the GS transgene is a GS polynucleotide encoding an Alfalfa derived GS1 (i.e., SEQ ID NO: 4) or an *Arabidopsis* derived GS1 (SEQ ID NO: 7). The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 1; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 1, and encoding a polypeptide having GPT activity; a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity; and a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2 truncated at its amino terminus by between 30 to 56 amino acid residues, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity. The GS1 transgene may be encoded by the polynucleotide of SEQ ID NO: 3 or SEQ ID NO: 6 or a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 3 or SEQ ID NO: 6, and encoding a polypeptide having GPT activity; and a nucleotide sequence encoding the polypeptide of SEQ ID NO: 4 or 7, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GS activity.

[0089] In another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Grape derived GPT, such as the Grape GPTs of SEQ ID NO: 9 and SEQ ID NO: 31, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 8; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 8, and encoding a polypeptide having GPT activity; a nucleotide sequence encoding the polypeptide of SEQ ID NO: 9 or SEQ ID NO: 31, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0090] In yet another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Rice derived GPT, such as the Rice GPTs of SEQ ID NO: 11 and SEQ ID NO: 32, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 10; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 10, and encoding a polypeptide having GPT activity; a nucleotide

sequence encoding the polypeptide of SEQ ID NO: 11 or SEQ ID NO: 32, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0091] In yet another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Soybean derived GPT, such as the Soybean GPTs of SEQ ID NO: 13, SEQ ID NO: 33 or SEQ ID NO: 33 with a further Isoleucine at the N-terminus of the sequence, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 12; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 12, and encoding a polypeptide having GPT activity; a nucleotide sequence encoding the polypeptide of SEQ ID NO: 13 or SEQ ID NO: 33 or SEQ ID NO: 33 with a further Isoleucine at the N-terminus of the sequence, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0092] In yet another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Barley derived GPT, such as the Barley GPTs of SEQ ID NO: 15 and SEQ ID NO: 34, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 14; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 10, and encoding a polypeptide having GPT activity; a nucleotide sequence encoding the polypeptide of SEQ ID NO: 15 or SEQ ID NO: 34, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0093] In yet another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Zebra fish derived GPT, such as the Zebra fish GPTs of SEQ ID NO: 17 and SEQ ID NO: 35, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by the nucleotide sequence of SEQ ID NO: 16; a nucleotide sequence having at least 75% and more preferably at least 80% identity to SEQ ID NO: 16, and encoding a polypeptide having GPT activity; a nucleotide sequence encoding the polypeptide of SEQ ID NO: 17 or SEQ ID NO: 35, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0094] In yet another specific embodiment, the GPT transgene is a GPT polynucleotide encoding a Bamboo derived GPT, such as the Bamboo GPT of SEQ ID NO: 36, and the GS transgene is a GS1 polynucleotide. The GPT transgene may be encoded by a nucleotide sequence encoding the polypeptide of SEQ ID NO: 36, or a polypeptide having at least 75% and more preferably at least 80% sequence identity thereto which has GPT activity.

[0095] Other GPT polynucleotides suitable for use as GPT transgenes in the practice of the invention may be obtained by various means, as will be appreciated by one skilled in the art, tested for the ability to direct the expression of a GPT with GPT activity in a recombinant expression system (i.e., *E. coli* (see Examples 20-23), in a transient in planta expression system (see Example 19), or in a transgenic plant (see Examples 1-18).

Transgene Constructs/Expression Vectors

[0096] In order to generate the transgenic plants of the invention, the gene coding sequence for the desired transgene (s) must be incorporated into a nucleic acid construct (also interchangeably referred to herein as a/an (transgene) expression vector, expression cassette, expression construct or expressible genetic construct), which can direct the expression of the transgene sequence in transformed plant cells. Such nucleic acid constructs carrying the transgene(s) of interest may be introduced into a plant cell or cells using a number of methods known in the art, including but not limited to electroporation, DNA bombardment or biolistic approaches, microinjection, and via the use of various DNA-based vectors such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* vectors. Once introduced into the transformed plant cell, the nucleic acid construct may direct the expression of the incorporated transgene(s) (i.e., GPT), either in a transient or stable fashion. Stable expression is preferred, and is achieved by utilizing plant transformation vectors which are able to direct the chromosomal integration of the transgene construct. Once a plant cell has been successfully transformed, it may be cultivated to regenerate a transgenic plant.

[0097] A large number of expression vectors suitable for driving the constitutive or induced expression of inserted genes in transformed plants are known. In addition, various transient expression vectors and systems are known. To a large extent, appropriate expression vectors are selected for use in a particular method of gene transformation (see, infra). Broadly speaking, a typical plant expression vector for generating transgenic plants will comprise the transgene of interest under the expression regulatory control of a promoter, a selectable marker for assisting in the selection of transformants, and a transcriptional terminator sequence.

[0098] More specifically, the basic elements of a nucleic acid construct for use in generating the transgenic plants of the invention are: a suitable promoter capable of directing the functional expression of the transgene(s) in a transformed plant cell, the transgene (s) (i.e., GPT coding sequence) operably linked to the promoter, preferably a suitable transcription termination sequence (i.e., nopaline synthetic enzyme gene terminator) operably linked to the transgene, and sometimes other elements useful for controlling the expression of the transgene, as well as one or more selectable marker genes suitable for selecting the desired transgenic product (i.e., antibiotic resistance genes).

[0099] As *Agrobacterium tumefaciens* is the primary transformation system used to generate transgenic plants, there are numerous vectors designed for *Agrobacterium* transformation. For stable transformation, *Agrobacterium* systems utilize "binary" vectors that permit plasmid manipulation in both *E. coli* and *Agrobacterium*, and typically contain one or more selectable markers to recover transformed plants (Hellens et al., 2000, *Technical focus: A guide to Agrobacterium binary Ti vectors*. Trends Plant Sci 5:446-451). Binary vectors for use in *Agrobacterium* transformation systems typically comprise the borders of T-DNA, multiple cloning sites, replication functions for *Escherichia coli* and *A. tumefaciens*, and selectable marker and reporter genes.

[0100] So-called "super-binary" vectors provide higher transformation efficiencies, and generally comprise additional virulence genes from a Ti (Komari et al., 2006, Methods Mol. Biol. 343: 15-41). Super binary vectors are typically used in plants which exhibit lower transformation efficiencies, such as cereals. Such additional virulence genes include without limitation virB, virE, and virG (Vain et al., 2004, *The effect of additional virulence genes on transformation efficiency, transgene integration and expression in rice plants*

using the pGreen/pSoup dual binary vector system. Transgenic Res. 13: 593-603; Srivatanakul et al., 2000, *Additional virulence genes influence transgene expression: transgene copy number, integration pattern and expression.* J. Plant Physiol. 157, 685-690; Park et al., 2000, Shorter T-DNA or additional virulence genes improve *Agrobacterium-mediated transformation.* Theor. Appl. Genet. 101, 1015-1020; Jin et al., 1987, *Genes responsible for the supervirulence phenotype of Agrobacterium tumefaciens A*281. J. Bacteriol. 169: 4417-4425).

[0101] In the embodiments exemplified herein (see Examples, infra), expression vectors which place the inserted transgene(s) under the control of the constitutive CaMV 35S promoter and the RuBisCo promoter are employed. A number of expression vectors which utilize the CaMV 35S and RuBsCo promoter are known and/or commercially available and/or derivable using ordinary skill in the art. Additionally, numerous promoters suitable for directing the expression of the transgene are known and may be used in the practice of the invention, as further described, infra.

Plant Promoters

[0102] A large number of promoters which are functional in plants are known in the art. In constructing GPT and GS transgene constructs, the selected promoter(s) may be constitutive, non-specific promoters such as the Cauliflower Mosaic Virus 35S ribosomal promoter (CaMV 35S promoter), which is widely employed for the expression of transgenes in plants. Examples of other strong constitutive promoters include without limitation the rice actin 1 promoter, the CaMV 19S promoter, the Ti plasmid nopaline synthase promoter, the alcohol dehydrogenase promoter and the sucrose synthase promoter.

[0103] Alternatively, in some embodiments, it may be desirable to select a promoter based upon the desired plant cells to be transformed by the transgene construct, the desired expression level of the transgene, the desired tissue or subcellular compartment for transgene expression, the developmental stage targeted, and the like.

[0104] For example, when expression in photosynthetic tissues and compartments is desired, a promoter of the ribulose bisphosphate carboxylase (RuBisCo) gene may be employed. In the Examples which follow, expressible nucleic acid constructs comprising GPT and GS1 transgenes under the control of a tomato RuBisCo promoter were prepared and used in the generation of transgenic plants or to assay for GPT activity in planta or in *E. coli.*

[0105] When the expression in seeds is desired, promoters of various seed storage protein genes may be employed. For expression in fruits, a fruit-specific promoter such as tomato 2A11 may be used. Examples of other tissue specific promoters include the promoters encoding lectin (Vodkin et al., 1983, Cell 34:1023-31; Lindstrom et al., 1990, Developmental Genetics 11:160-167), corn alcohol dehydrogenase 1 (Vogel et al, 1989, J. Cell. Biochem. (Suppl. 0) 13:Part D; Dennis et al., 1984, Nucl. Acids Res., 12(9): 3983-4000), corn light harvesting complex (Simpson, 1986, Science, 233: 34-38; Bansal et al., 1992, Proc. Natl. Acad. Sci. USA, 89: 3654-3658), corn heat shock protein (Odell et al., 1985, Nature, 313: 810-812; Rochester et al., 1986, EMBO J., 5: 451-458), pea small subunit RuBP carboxylase (Poulsen et al., 1986, Mol. Gen. Genet., 205(2): 193-200; Cashmore et al., 1983, Gen. Eng. Plants, Plenum Press, New York, pp 29-38), Ti plasmid mannopine synthase and Ti plasmid nopaline synthase (Langridge et al., 1989, Proc. Natl. Acad. Sci. USA, 86: 3219-3223), petunia chalcone isomerase (Van Tunen et al., 1988, EMBO J. 7(5): 1257-1263), bean glycine rich protein 1 (Keller et al., 1989, EMBO J. 8(5): 1309-1314), truncated CaMV 35s (Odell et al., 1985, supra), potato patatin (Wenzler et al., 1989, Plant Mol. Biol. 12: 41-50), root cell (Conkling et al., 1990, Plant Physiol. 93: 1203-1211), maize zein (Reina et al., 1990, Nucl. Acids Res. 18(21): 6426; Kriz et al., 1987, Mol. Gen. Genet. 207(1): 90-98; Wandelt and Feix, 1989, Nuc. Acids Res. 17(6): 2354; Langridge and Feix, 1983, Cell 34: 1015-1022; Reina et al., 1990, Nucl. Acids Res. 18(21): 6426), globulin-1 (Belanger and Kriz, 1991, Genetics 129: 863-872), α-tubulin (Carpenter et al., 1992, Plant Cell 4(5): 557-571; Uribe et al., 1998, Plant Mol. Biol. 37(6): 1069-1078), cab (Sullivan, et al., 1989, Mol. Gen. Genet. 215(3): 431-440), PEPCase (Hudspeth and Grula, 1989, Plant Mol. Biol. 12: 579-589), R gene complex (Chandler et al., 1989, The Plant Cell 1: 1175-1183), chalcone synthase (Franken et al., 1991, EMBO J. 10(9): 2605-2612) and glutamine synthetase promoters (U.S. Pat. No. 5,391,725; Edwards et al., 1990, Proc. Natl. Acad. Sci. USA 87: 3459-3463; Brears et al., 1991, Plant J. 1(2): 235-244).

[0106] In addition to constitutive promoters, various inducible promoter sequences may be employed in cases where it is desirable to regulate transgene expression as the transgenic plant regenerates, matures, flowers, etc. Examples of such inducible promoters include promoters of heat shock genes, protection responding genes (i.e., phenylalanine ammonia lyase; see, for example Bevan et al., 1989, EMBO J. 8(7): 899-906), wound responding genes (i.e., cell wall protein genes), chemically inducible genes (i.e., nitrate reductase, chitinase) and dark inducible genes (i.e., asparagine synthetase; see, for example U.S. Pat. No. 5,256,558). Also, a number of plant nuclear genes are activated by light, including gene families encoding the major chlorophyll a/b binding proteins (cab) as well as the small subunit of ribulose-1,5-bisphosphate carboxylase (rbcS) (see, for example, Tobin and Silverthorne, 1985, Annu. Rev. Plant Physiol. 36: 569-593; Dean et al., 1989, Annu. Rev. Plant Physiol. 40: 415-439.).

[0107] Other inducible promoters include ABA- and turgor-inducible promoters, the auxin-binding protein gene promoter (Schwob et al., 1993, Plant J. 4(3): 423-432), the UDP glucose flavonoid glycosyl-transferase gene promoter (Ralston et al., 1988, Genetics 119(1): 185-197); the MPI proteinase inhibitor promoter (Cordero et al., 1994, Plant J. 6(2): 141-150), the glyceraldehyde-3-phosphate dehydrogenase gene promoter (Kohler et al., 1995, Plant Mol. Biol. 29(6): 1293-1298; Quigley et al., 1989, J. Mol. Evol. 29(5): 412-421; Martinez et al., 1989, J. Mol. Biol. 208(4): 551-565) and light inducible plastid glutamine synthetase gene from pea (U.S. Pat. No. 5,391,725; Edwards et al., 1990, supra).

[0108] For a review of plant promoters used in plant transgenic plant technology, see Potenza et al., 2004, In Vitro Cell. Devel. Biol-Plant, 40(1): 1-22. For a review of synthetic plant promoter engineering, see, for example, Venter, M., 2007, Trends Plant Sci., 12(3): 118-124.

Glutamine Phenylpyruvate Transaminase (GPT) Transgene

[0109] The present invention discloses for the first time that plants contain a glutamine phenylpyruvate transaminase (GPT) enzyme which is directly functional in the synthesis of the signal metabolite 2-hydroxy-5-oxoproline. Until now, no plant glutamine phenylpyruvate transaminase with a defined function has been described. Applicants have isolated and

tested GPT polynucleotide coding sequences derived from several plant and animal species, and have successfully incorporated the gene into heterologous transgenic host plants which exhibit markedly improved growth characteristics, including faster growth, higher foliar protein content, increased glutamine synthetase activity in foliar tissue, and faster CO_2 fixation rates.

[0110] In the practice of the invention, the GPT gene functions as one of at least two transgenes incorporated into the transgenic plants of the invention, the other being the glutamine sythetase gene (see infra).

[0111] It is expected that all plant species contain a GPT which functions in the same metabolic pathway, involving the biosynthesis of the signal metabolite 2-hydroxy-5-oxoproline. Thus, in the practice of the invention, any plant gene encoding a GPT homolog or functional variants thereof may be useful in the generation of transgenic plants of this invention. Moreover, given the structural similarity between various plant GPT protein structures and the putative (and biologically active) GPT homolog from *Danio rerio* (Zebra fish) (see Example 22), other non-plant GPT homologs may be used in preparing GPT transgenes for use in generating the transgenic plants of the invention.

[0112] When individually compared (by BLAST alignment) to the *Arabidopsis* mature protein sequence provided in SEQ ID NO: 30, the following sequence identities and homologies (BLAST "positives", including similar amino acids) were obtained for the following mature GPT protein sequences:

[SEQ ID] or FIG. NO.	ORIGIN	% IDENTITY	% POSITIVE
[31]	Grape	84	93
[32]	Rice	83	91
[33]	Soybean	83	93
[34]	Barley	82	91
[35]	Zebra fish	83	92
[36]	Bamboo	81	90
FIG. 2	Corn	79	90
FIG. 2	Castor	84	93
FIG. 2	Poplar	85	93

[0113] Underscoring the conserved nature of the structure of the GPT protein across most plant species, the conservation seen within the above plant species extends to the non-human putative GPTs from Zebra fish and *Chlamydomonas*. In the case of Zebra fish, the extent of identity is very high (83% amino acid sequence identity with the mature *Arabidopsis* GPT of SEQ ID NO: 30, and 92% homologous taking similar amino acid residues into account). The Zebra fish mature GPT was confirmed by expressing it in *E. coli* and demonstrating biological activity (synthesis of 2-oxoglutaramate).

[0114] In order to determine whether putative GPT homologs would be suitable for generating the growth-enhanced transgenic plants of the invention, one need initially express the coding sequence thereof in *E. coli* or another suitable host and determine whether the 2-oxoglutaramate signal metabolite is synthesized at increased levels (see Examples 19-23). Where such an increase is demonstrated, the coding sequence may then be introduced into both homologous plant hosts and heterologous plant hosts, and growth characteristics evaluated. Any assay that is capable of detecting 2-oxoglutaramate with specificity may be used for this purpose, including without limitation the NMR and HPLC assays described in Example 2, infra. In addition, assays which measure GPT activity directly may be employed, such as the GPT activity assay described in Example 7.

[0115] Any plant GPT with 2-oxoglutaramate synthesis activity may be used to transform plant cells in order to generate transgenic plants of the invention. There appears to be a high level of structural homology among plant species, which appears to extend beyond plants, as evidenced by the close homology between various plant GPT proteins and the putative Zebra fish GPT homolog. Therefore, various plant GPT genes may be used to generate growth-enhanced transgenic plants in a variety of heterologous plant species. In addition, GPT transgenes expressed in a homologous plant would be expected to result in the desired enhanced-growth characteristics as well (i.e., rice glutamine transaminase over-expressed in transgenic rice plants), although it is possible that regulation within a homologous cell may attenuate the expression of the transgene in some fashion that may not be operable in a heterologous cell.

Glutamine Synthetase (GS) Transgene:

[0116] In the practice of the invention, the glutamine synthetase (GS) gene functions as one of at least two transgenes incorporated into the transgenic plants of the invention (GPT being the other of the two).

[0117] Glutamine synthetase plays a key role in nitrogen metabolism in plants, as well as in animals and bacteria. The GS enzyme catalyzes the addition of ammonium to glutamate to synthesize glutamine in an ATP-dependent reaction. GS enzymes from assorted species show highly conserved amino acid residues considered to be important for active site function, indicating that GS enzymes function similarly (for review, see Eisenberg et al., Biochimica et Biophysica Acta, 1477:122 145, 2000).

[0118] GS is distributed in different subcellular locations (chloroplast and cytoplasm) and is found in various plant tissues, including leaf, root, shoot, seeds and fruits. There are two major isoforms of plant GS: the cystolic isoform (GS1) and the plastidic (chloroplastic) isoform (GS2). GS2 is principally found in leaf tissue and functions in the assimilation of ammonia produced by photorespiration or by nitrate reduction. GS1 is mainly found in leaf and root tissue, typically exists in a number of different isoforms in higher plants, and functions to assimilate ammonia produced by all other physiological processes (Coruzzi, 1991, Plant Science 74: 145-155; McGrath and Coruzzi, 1991, Plant J. 1(3): 275-280; Lam et al., 1996, Ann. Rev. Plant Physiol. Plant Mol. Biol. 47: 569-593; Stitt, 1999, Curr. Op. Plant Biol. 2: 178-186; Oliveira et al., 2001, Brazilian J. Med. Biol. Res. 34: 567-575). Multiple GS genes are associated with a complex promoter repertoire which enable the expression of GS in an organ and tissue specific manner, as well as in an environmental factor-dependent manner.

[0119] Plant glutamine synthetase consists of eight subunits, and the native enzyme in plants has a molecular mass ranging from 320 to 380 kD, each subunit having a molecular mass of between 38 and 45 kD. The GS1 genes of several plants, especially legumes, have been cloned and sequenced (Tischer et al., 1986, Mol Gen Genet. 203: 221-229; Gebhardt et al., 1986, EMBO J. 5: 1429-1435; Tingey et al., 1987, EMBO J. 6: 1-9; Tingey et al., 1988, J Biol. Chem. 263: 9651-9657; Bennett et al., 1989, Plant Mol. Biol. 12: 553-

565; Boron and Legocki, 1993, Gene 136: 95-102; Roche et al., 1993, Plant Mol. Biol. 22: 971-983; Marsolier et al., 1995, Plant Mol. Biol. 27: 1-15; Temple et al., 1995, Mol Plant-Microbe Interact. 8: 218-227). All have been found to be encoded by nuclear genes (for review, see, Morey et al., 2002, Plant Physiol. 128(1): 182-193).

[0120] Chloroplastic GS2 appears to be encoded by a single gene, while various cystoloic GS1 isoforms are encoded within multigene families (Tingey et al., 1987, supra; Sakamoto et al., 1989, Plant Mol. Biol. 13: 611-614; Brears et al, 1991, supra; Li et al., 1993, Plant Mol. Biol., 23:401-407; Dubois et al., 1996, Plant Mol. Biol., 31:803-817; Lam et al., 1996, supra). GS1 multigene families appear to encode different subunits which may combine to form homo- or heterooctamers, and the different members show a unique expression pattern suggesting that the gene members are differentially regulated, which may relate to the various functional roles of glutamine synthetase plays in overall nitrogen metabolism (Gebhardt et al., 1986, supra; Tingey et al., 1987, supra; Bennett et al., 1989, supra; Walker and Coruzzi, 1989, supra; Peterman and Goodman, 1991, Mol Gen Genet. 1991; 330:145-154; Marsolier et al., 1995, supra; Temple et al., 1995, supra; Dubois et al., 1996, supra).

[0121] In one embodiment, a GS1 gene coding sequence is employed to generate GS transgene constructs. In particular embodiments, further described in the Examples, infra, the Alfalfa or *Arabidopsis* GS1 gene coding sequence is used to generate a transgene construct that may be used to generate a transgenic plant expressing the GS1 transgene. As an example, such a construct may be used to transform *Agrobacteria*. The transformed *Agrobacteria* are then used to generate T_0 transgenic plants. Example 5 demonstrates the generation of T_0 GS1 transgenic tobacco plants using this approach. Similarly, Examples 6 and 17 demonstrates the generation of T_0 GS1 transgenic tomato plants, Example 8 demonstrates the generation of T_0 GS1 transgenic pepper plants, Examples 9 and 10 demonstrate the generation of T_0 GS1 transgenic bean plants, Examples 11 and 12 demonstrate the generation of T_0 GS1 transgenic cowpea plants, Example 13 demonstrates the generation of T_0 GS1 transgenic alfalfa plants, Example 14 demonstrates the generation of T_0 GS1 transgenic cantaloupe plants, Example 15 demonstrates the generation of T_0 GS1 transgenic pumpkin plants, Example 16 demonstrates the generation of T_0 GS1 transgenic *Arabidopsis* plants, and Example 18 demonstrates the generation of T_0 GS1 transgenic Cantaloupe plants.

Transcription Terminators:

[0122] In preferred embodiments, a 3' transcription termination sequence is incorporated downstream of the transgene in order to direct the termination of transcription and permit correct polyadenylation of the mRNA transcript. Suitable transcription terminators are those which are known to function in plants, including without limitation, the nopaline synthase (NOS) and octopine synthase (OCS) genes of *Agrobacterium tumefaciens*, the T7 transcript from the octopine synthase gene, the 3' end of the protease inhibitor I or II genes from potato or tomato, the CaMV 35S terminator, the tml terminator and the pea rbcS E9 terminator. In addition, a gene's native transcription terminator may be used. In specific embodiments, described by way of the Examples, infra, the nopaline synthase transcription terminator is employed.

Selectable Markers:

[0123] Selectable markers are typically included in transgene expression vectors in order to provide a means for selecting transformants. While various types of markers are available, various negative selection markers are typically utilized, including those which confer resistance to a selection agent that inhibits or kills untransformed cells, such as genes which impart resistance to an antibiotic (such as kanamycin, gentamycin, anamycin, hygromycin and hygromycinB) or resistance to a herbicide (such as sulfonylurea, gulfosinate, phosphinothricin and glyphosate). Screenable markers include, for example, genes encoding β-glucuronidase (Jefferson, 1987, Plant Mol. Biol. Rep 5: 387-405), genes encoding luciferase (Ow et al., 1986, Science 234: 856-859) and various genes encoding proteins involved in the production or control of anthocyanin pigments (See, for example, U.S. Pat. No. 6,573,432). The *E. coli* glucuronidase gene (gus, gusA or uidA) has become a widely used selection marker in plant transgenics, largely because of the glucuronidase enzyme's stability, high sensitivity and ease of detection (e.g., fluorometric, spectrophotometric, various histochemical methods). Moreover, there is essentially no detectable glucuronidase in most higher plant species.

Transformation Methodologies and Systems:

[0124] Various methods for introducing the transgene expression vector constructs of the invention into a plant or plant cell are well known to those skilled in the art, and any capable of transforming the target plant or plant cell may be utilized.

[0125] *Agrobacterium*-mediated transformation is perhaps the most common method utilized in plant transgenics, and protocols for *Agrobacterium*-mediated transformation of a large number of plants are extensively described in the literature (see, for example, *Agrobacterium Protocols*, Wan, ed., Humana Press, 2nd edition, 2006). *Agrobacterium tumefaciens* is a Gram negative soil bacteria that causes tumors (Crown Gall disease) in a great many dicot species, via the insertion of a small segment of tumor-inducing DNA ("T-DNA", 'transfer DNA') into the plant cell, which is incorporated at a semi-random location into the plant genome, and which eventually may become stably incorporated there. Directly repeated DNA sequences, called T-DNA borders, define the left and the right ends of the T-DNA. The T-DNA can be physically separated from the remainder of the Ti-plasmid, creating a 'binary vector' system.

[0126] *Agrobacterium* transformation may be used for stably transforming dicots, monocots, and cells thereof (Rogers et al., 1986, Methods Enzymol., 118: 627-641; Hernalsteen et al., 1984, EMBO J., 3: 3039-3041; Hoykass-Van Slogteren et al., 1984, Nature, 311: 763-764; Grimsley et al., 1987, Nature 325: 167-1679; Boulton et al., 1989, Plant Mol. Biol. 12: 31-40; Gould et al., 1991, Plant Physiol. 95: 426-434). Various methods for introducing DNA into *Agrobacteria* are known, including electroporation, freeze/thaw methods, and triparental mating. The most efficient method of placing foreign DNA into *Agrobacterium* is via electroporation (Wise et al., 2006, *Three Methods for the Introduction of Foreign DNA into Agrobacterium*, Methods in Molecular Biology, vol. 343: *Agrobacterium* Protocols, 2/e, volume 1; Ed., Wang, Humana Press Inc., Totowa, N.J., pp. 43-53). In addition, given that a large percentage of T-DNAs do not integrate, *Agrobacterium*-mediated transformation may be used to obtain transient expression of a transgene via the transcriptional competency of unincorporated transgene construct molecules (Helens et al., 2005, Plant Methods 1:13).

[0127] A large number of *Agrobacterium* transformation vectors and methods have been described (Karimi et al., 2002, Trends Plant Sci. 7(5): 193-5), and many such vectors may be obtained commercially (for example, Invitrogen, Carlsbad, Calif.). In addition, a growing number of "open-source" *Agrobacterium* transformation vectors are available (for example, pCambia vectors; Cambia, Canberra, Australia). See, also, subsection herein on TRANSGENE CONSTRUCTS, supra. In a specific embodiment described further in the Examples, a pMON316-based vector was used in the leaf disc transformation system of Horsch et. al. (Horsch et al., 1995, Science 227:1229-1231) to generate growth enhanced transgenic tobacco and tomato plants.

[0128] Other commonly used transformation methods that may be employed in generating the transgenic plants of the invention include, without limitation microprojectile bombardment, or biolistic transformation methods, protoplast transformation of naked DNA by calcium, polyethylene glycol (PEG) or electroporation (Paszkowski et al., 1984, EMBO J. 3: 2727-2722; Potrykus et al., 1985, Mol. Gen. Genet. 199: 169-177; Fromm et al., 1985, Proc. Nat. Acad. Sci. USA 82: 5824-5828; Shimamoto et al., 1989, Nature, 338: 274-276.

[0129] Biolistic transformation involves injecting millions of DNA-coated metal particles into target cells or tissues using a biolistic device (or "gene gun"), several kinds of which are available commercially. Once inside the cell, the DNA elutes off the particles and a portion may be stably incorporated into one or more of the cell's chromosomes (for review, see Kikkert et al., 2005, *Stable Transformation of Plant Cells by Particle Bombardment/Biolistics*, in: Methods in Molecular Biology, vol. 286: Transgenic Plants: Methods and Protocols, Ed. L. Peña, Humana Press Inc., Totowa, N.J.).

[0130] Electroporation is a technique that utilizes short, high-intensity electric fields to permeabilize reversibly the lipid bilayers of cell membranes (see, for example, Fisk and Dandekar, 2005, *Introduction and Expression of Transgenes in Plant Protoplasts*, in: Methods in Molecular Biology, vol. 286: Transgenic Plants: Methods and Protocols, Ed. L. Peña, Humana Press Inc., Totowa, N.J., pp. 79-90; Fromm et al., 1987, *Electroporation of DNA and RNA into plant protoplasts*, in Methods in Enzymology, Vol. 153, Wu and Grossman, eds., Academic Press, London, UK, pp. 351-366; Joersbo and Brunstedt, 1991, *Electroporation: mechanism and transient expression, stable transformation and biological effects in plant protoplasts*. Physiol. Plant. 81, 256-264; Bates, 1994, *Genetic transformation of plants by protoplast electroporation*. Mol. Biotech. 2: 135-145; Dillen et al., 1998, *Electroporation-mediated DNA transfer to plant protoplasts and intact plant tissues for transient gene expression assays*, in Cell Biology, Vol. 4, ed., Celis, Academic Press, London, UK, pp. 92-99). The technique operates by creating aqueous pores in the cell membrane, which are of sufficiently large size to allow DNA molecules (and other macromolecules) to enter the cell, where the transgene expression construct (as T-DNA) may be stably incorporated into plant genomic DNA, leading to the generation of transformed cells that can subsequently be regenerated into transgenic plants.

[0131] Newer transformation methods include so-called "floral dip" methods, which offer the promise of simplicity, without requiring plant tissue culture, as is the case with all other commonly used transformation methodologies (Bent et al., 2006, *Arabidopsis thaliana Floral Dip Transformation Method*, Methods Mol Biol, vol. 343: *Agrobacterium Protocols,* 2/e, volume 1; Ed., Wang, Humana Press Inc., Totowa, N.J., pp. 87-103; Clough and Bent, 1998, *Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana*, Plant J. 16: 735-743). However, with the exception of *Arabidopsis*, these methods have not been widely used across a broad spectrum of different plant species. Briefly, floral dip transformation is accomplished by dipping or spraying flowering plants in with an appropriate strain of *Agrobacterium tumefaciens*. Seeds collected from these T_0 plants are then germinated under selection to identify transgenic T_1 individuals. Example 16 demonstrated floral dip inoculation of *Arabidopsis* to generate transgenic *Arabidopsis* plants.

[0132] Other transformation methods include those in which the developing seeds or seedlings of plants are transformed using vectors such as *Agrobacterium* vectors. For example, as exemplified in Example 8, such vectors may be used to transform developing seeds by injecting a suspension or mixture of the vector (i.e., *Agrobacteria*) directly into the seed cavity of developing pods (i.e., pepper pods, bean pods, pea pods and the like). Seedlings may be transformed as described for Alfalfa in Example 13. Germinating seeds may be transformed as described for Camelina in Example 18. Intra-fruit methods, in which the vector is injected into fruit or developing fruit, may be used as described for Cantaloupe melons in Example 14 and pumpkins in Example 15.

[0133] Still other transformation methods include those in which the flower structure is targeted for vector inoculation, such as the flower inoculation methods described for beans in Examples 9 and 10, peas in Examples 11 and 12 and tomatoes in Example 17.

[0134] In addition, although transgenes are most commonly inserted into the nuclear DNA of plant cells, trangenes may also be inserted into plastidic DNA (i.e., into the plastome of the chloroplast). In most flowering plants, plastids do not occur in the pollen cells, and therefore transgenic DNA incorporated within a plastome will not be passed on through propagation, thereby restricting the trait from migrating to wild type plants. Plastid transformation, however, is more complex than cell nucleus transformation, due to the presence of many thousands of plastomes per cell (as high as 10,000).

[0135] Transplastomic lines are genetically stable only if all plastid copies are modified in the same way, i.e. uniformly. This is typically achieved through repeated regeneration under certain selection conditions to eliminate untransformed plastids, by segregating transplastomic and untransformed plastids, resulting in the selection of homoplasmic cells carrying the transgene construct and the selectable marker stably integrated therein. Plastid transformation has been successfully performed in various plant species, including tobacco, potatoes, oilseed rape, rice and *Arabidopsis*.

[0136] To generate transplastomic lines carrying GPT and GS transgenes, the transgene expression cassette is inserted into flanking sequences from the plastome. Using homologous recombination, the transgene expression cassette becomes integrated into the plastome via a natural recombination process. The basic DNA delivery techniques for plastid transformation include particle bombardment of leaves or polyethylene glycol-mediated DNA transformation of protoplasts. Transplastomic plants carrying transgenes in the plastome may be expressed at very high levels, due to the fact that many plastids (i.e., chloroplasts) per cell, each carrying many copies of the plastome. This is particularly the case in foliar tissue, where a single mature leaf cell may contain over 10,000 copies of the plastome. Following a successful trans-

formation of the plastome, the transplastomic events carry the transgene on every copy of the plastid genetic material. This can result in the transgene expression levels representing as much as half of the total protein produced in the cell.

[0137] Plastid transformation methods and vector systems are described, for example, in recent U.S. Pat. Nos. 7,528,292; 7,371,923; 7,235,711; and, 7,193,131. See also U.S. Pat. Nos. 6,680,426 and 6,642,053.

[0138] The foregoing plant transformation methodologies may be used to introduce transgenes into a number of different plant cells and tissues, including without limitation, whole plants, tissue and organ explants including chloroplasts, flowering tissues and cells, protoplasts, meristem cells, callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells, tissue cultured cells of any of the foregoing, any other cells from which a fertile regenerated transgenic plant may be generated. Callus is initiated from tissue sources including, but not limited to, immature embryos, seedling apical meristems, microspores and the like. Cells capable of proliferating as callus are also recipient cells for genetic transformation.

[0139] Methods of regenerating individual plants from transformed plant cells, tissues or organs are known and are described for numerous plant species.

[0140] As an illustration, transformed plantlets (derived from transformed cells or tissues) are cultured in a root-permissive growth medium supplemented with the selective agent used in the transformation strategy (i.e., and antibiotic such as kanamycin). Once rooted, transformed plantlets are then transferred to soil and allowed to grow to maturity. Upon flowering, the mature plants are preferably selfed (self-fertilized), and the resultant seeds harvested and used to grow subsequent generations. Examples 3-6 describe the regeneration of transgenic tobacco and tomato plants.

[0141] T_0 transgenic plants may be used to generate subsequent generations (e.g., T_1, T_2, etc.) by selfing of primary or secondary transformants, or by sexual crossing of primary or secondary transformants with other plants (transformed or untransformed). For example, as described in Example 7, infra, individual plants over expressing the Alfalfa GS1 gene and outperforming wildtype plants were crossed with individual plants over-expressing the *Arabidopsis* GPT gene and outperforming wildtype plants, by simple sexual crossing using manual pollen transfer. Reciprocal crosses were made such that each plant served as the male in a set of crosses and each plant served as the female in a second set of crosses. During the mature plant growth stage, the plants are typically examined for growth phenotype, CO_2 fixation rate, etc. (see following subsection).

Selection of Growth-Enhanced Transgenic Plants:

[0142] Transgenic plants may be selected, screened and characterized using standard methodologies. The preferred transgenic plants of the invention will exhibit one or more phenotypic characteristics indicative of enhanced growth and/or other desirable agronomic properties. Transgenic plants are typically regenerated under selective pressure in order to select transformants prior to creating subsequent transgenic plant generations. In addition, the selective pressure used may be employed beyond T_0 generations in order to ensure the presence of the desired transgene expression construct or cassette.

[0143] T_0 transformed plant cells, calli, tissues or plants may be identified and isolated by selecting or screening for the genetic composition of and/or the phenotypic characteristics encoded by marker genes contained in the transgene expression construct used for the transformation. For example, selection may be conducted by growing potentially-transformed plants, tissues or cells in a growth medium containing a repressive amount of antibiotic or herbicide to which the transforming genetic construct can impart resistance. Further, the transformed plant cells, tissues and plants can be identified by screening for the activity of marker genes (i.e., β-glucuronidase) which may be present in the transgene expression construct.

[0144] Various physical and biochemical methods may be employed for identifying plants containing the desired transgene expression construct, as is well known. Examples of such methods include Southern blot analysis or various nucleic acid amplification methods (i.e., PCR) for identifying the transgene, transgene expression construct or elements thereof, Northern blotting, S1 RNase protection, reverse transcriptase PCR (RT-PCR) amplification for detecting and determining the RNA transcription products; and protein gel electrophoresis, Western blotting, immunoprecipitation, enzyme immunoassay, and the like may be used for identifying the protein encoded and expressed by the transgene.

[0145] In another approach, expression levels of genes, proteins and/or metabolic compounds that are know to be modulated by transgene expression in the target plant may be used to identify transformants. In one embodiment of the present invention, increased levels of the signal metabolite 2-oxoglutaramate may be used to screen for desirable transformants, as exemplified in the Examples. Similarly, increased levels of GPT and/or GS activity may be assayed, as exemplified in the Examples.

[0146] Ultimately, the transformed plants of the invention may be screened for enhanced growth and/or other desirable agronomic characteristics. Indeed, some degree of phenotypic screening is generally desirable in order to identify transformed lines with the fastest growth rates, the highest seed yields, etc., particularly when identifying plants for subsequent selfing, cross-breeding and back-crossing. Various parameters may be used for this purpose, including without limitation, growth rates, total fresh weights, dry weights, seed and fruit yields (number, weight), seed and/or seed pod sizes, seed pod yields (e.g., number, weight), leaf sizes, plant sizes, increased flowering, time to flowering, overall protein content (in seeds, fruits, plant tissues), specific protein content (i.e., GS), nitrogen content, free amino acid, and specific metabolic compound levels (i.e., 2-oxoglutaramate). Generally, these phenotypic measurements are compared with those obtained from a parental identical or analogous plant line, an untransformed identical or analogous plant, or an identical or analogous wild-type plant (i.e., a normal or parental plant). Preferably, and at least initially, the measurement of the chosen phenotypic characteristic(s) in the target transgenic plant is done in parallel with measurement of the same characteristic(s) in a normal or parental plant. Typically, multiple plants are used to establish the phenotypic desirability and/or superiority of the transgenic plant in respect of any particular phenotypic characteristic.

[0147] Preferably, initial transformants are selected and then used to generate T_1 and subsequent generations by selfing (self-fertilization), until the transgene genotype breeds true (i.e., the plant is homozygous for the transgene). In practice, this is accomplished by screening at each generation for the desired traits and selfing those individuals, often

repeatedly (i.e., 3 or 4 generations). As exemplified herein, transgenic plant lines propagated through at least one sexual generation (Tobacco, *Arabidopsis*, Tomato) demonstrated higher transgene product activities compared to lines that did not have the benefit of sexual reproduction and the concomitant increase in transgene copy number.

[0148] Stable transgenic lines may be crossed and backcrossed to create varieties with any number of desired traits, including those with stacked transgenes, multiple copies of a transgene, etc. Various common breeding methods are well know to those skilled in the art (see, e.g., Breeding Methods for Cultivar Development, Wilcox J. ed., American Society of Agronomy, Madison Wis. (1987)). Additionally, stable transgenic plants may be further modified genetically, by transforming such plants with further transgenes or additional copies of the parental transgene. Also contemplated are transgenic plants created by single transformation events which introduce multiple copies of a given transgene or multiple transgenes.

[0149] In another aspect, the invention provides transgenic plants characterized by increased nitrogen use efficiency. Nitrogen use efficiency may be expressed as plant yield per given amount of nitrogen. In the Examples provided herein, the transgene and control plants all received the same nutrient solutions in the same amounts. The transgenic plants were consistently characterized by higher yields, and thus have higher nitrogen use efficiencies.

[0150] In yet another aspect, the invention provides transgenic plants and seeds thereof with increased tolerance to high salt growth conditions. This aspect of the invention is exemplified by Example 24, which describes the germination of transgenic tobacco plant seeds in very high salt conditions (200 mM NaCl). While counterpart wild type tobacco seeds germinated at a rate of only about 10%, on average, the transgenic tobacco seeds achieved nearly the same rate of germination obtained under no salt conditions for both transgenic and wild type seeds, or about 92%.

EXAMPLES

[0151] Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the invention.

Example 1

Isolation of *Arabidopsis* Gluamine Phenylpyruvate Transaminase (GPT) Gene

[0152] In an attempt to locate a plant enzyme that is directly involved in the synthesis of the signal metabolite 2-oxoglutaramate, applicants hypothesized that the putative plant enzyme might bear some degree of structural relationship to a human protein that had been characterized as being involved in the synthesis of 2-oxoglutaramate. The human protein, glutamine transaminase K (E.C. 2.6.1.64) (also referred in the literature as cysteine conjugate β-lyase, kyneurenine aminotransferase, glutamine phenylpyruvate transaminase, and other names), had been shown to be involved in processing of cysteine conjugates of halogenated xenobiotics (Perry et al., 1995, FEBS Letters 360:277-280). Rather than having an activity involved in nitrogen assimilation, however, human cysteine conjugate β-lyase has a detoxifying activity in humans, and in animals (Cooper and Meister, 1977, supra). Nevertheless, the potential involvement of this protein in the synthesis of 2-oxoglutaramate was of interest.

[0153] Using the protein sequence of human cysteine conjugate β-lyase, a search against the TIGR *Arabidopsis* plant database of protein sequences identified one potentially related sequence, a polypeptide encoded by a partial sequence at the *Arabidopsis* gene locus at At1q77670, sharing approximately 36% sequence homology/identity across aligned regions.

[0154] The full coding region of the gene was then amplified from an *Arabidopsis* cDNA library (Stratagene) with the following primer pair:

[SEQ ID NO: 37]
5'-CCCATCGATGTACC TGGACATAAATGGTGTGATG-3'

[SEQ ID NO: 38]
5'-GATGGTACCTCAGACTTTTCTCTTAAGCTTCTGCTTC-3'

[0155] These primers were designed to incorporate Cla I (ATCGAT) and Kpn I (GGTACC) restriction sites to facilitate subsequent subcloning into expression vectors for generating transgenic plants. Takara ExTaq DNA polymerase enzyme was used for high fidelity PCR using the following conditions: initial denaturing 94° C. for 4 minutes, 30 cycles of 94° C. 30 second, annealing at 55° C. for 30 seconds, extension at 72° C. for 90 seconds, with a final extension of 72° C. for 7 minutes. The amplification product was digested with Cla I and Kpn I restriction enzymes, isolated from an agarose gel electrophoresis and ligated into vector pMon316 (Rogers, et. al. 1987 Methods in Enzymology 153:253-277) which contains the cauliflower mosaic virus (CaMV, also CMV) 35S constitutive promoter and the nopaline synthase (NOS) 3' terminator. The ligation product was transformed into DH5a cells and transformants sequenced to verify the insert.

[0156] A 1.3 kb cDNA was isolated and sequenced, and found to encode a full length protein of 440 amino acids in length, including a putative chloroplast signal sequence.

Example 2

Production of Biologically Active Recombinant *Arabidopsis* Glutamine Phenylpyruvate Transaminase (GPT)

[0157] To test whether the protein encoded by the cDNA isolated as described in Example 1, supra, is capable of catalyzing the synthesis of 2-oxoglutaramate, the cDNA was expressed in *E. coli*, purified, and assayed for its ability to synthesize 2-oxoglutaramate using a standard method.

NMR Assay for 2-Oxoglutaramate:

[0158] Briefly, the resulting purified protein was added to a reaction mixture containing 150 mM Tris-HCl, pH 8.5, 1 mM beta mercaptoethanol, 200 mM glutamine, 100 mM glyoxylate and 200 μM pyridoxal 5'-phosphate. The reaction mixture without added test protein was used as a control. Test and control reaction mixtures were incubated at 37° C. for 20 hours, and then clarified by centrifugation to remove precipitated material. Supernatants were tested for the presence and amount of 2-oxoglutaramate using ^{13}C NMR with authentic chemically synthesized 2-oxoglutaramate as a reference. The products of the reaction are 2-oxoglutaramate and glycine, while the substrates (glutamine and glyoxylate) diminish in abundance. The cyclic 2-oxoglutaramate gives rise to a dis-

tinctive signal allowing it to be readily distinguished from the open chain glutamine precursor.

HPLC Assay for 2-Oxoglutaramate:

[0159] An alternative assay for GPT activity uses HPLC to determine 2-oxoglutaramate production, following a modification of Calderon et al., 1985, J Bacteriol 161(2): 807-809. Briefly, a modified extraction buffer consisting of 25 mM Tris-HCl pH 8.5, 1 mM EDTA, 20 μM FAD, 10 mM Cysteine, and ~1.5% (v/v) Mercaptoethanol. Tissue samples from the test material (i.e., plant tissue) are added to the extraction buffer at approximately a 1/3 ratio (w/v), incubated for 30 minutes at 37° C., and stopped with 200 μl of 20% TCA. After about 5 minutes, the assay mixture is centrifuged and the supernatant used to quantify 2-oxoglutaramate by HPLC, using an ION-300 7.8 mm ID×30 cm L column, with a mobile phase in 0.01N H_2SO_4, a flow rate of approximately 0.2 ml/min, at 40° C. Injection volume is approximately 20 and retention time between about 38 and 39 minutes. Detection is achieved with 210 nm UV light.

Results Using NMR Assay:

[0160] This experiment revealed that the test protein was able to catalyze the synthesis of 2-oxoglutaramate. Therefore, these data indicate that the isolated cDNA encodes a glutamine phenylpyruvate transaminase that is directly involved in the synthesis of 2-oxoglutaramate in plants. Accordingly, the test protein was designated *Arabidopsis* glutamine phenylpyruvate transaminase, or "GPT".

[0161] The nucleotide sequence of the *Arabidopsis* GPT coding sequence is shown in the Table of Sequences, SEQ ID NO. 1. The translated amino acid sequence of the GPT protein is shown in SEQ ID NO. 2.

Example 3

Creation of Transgenic Tobacco Plants Over-Expressing *Arabidopsis* GPT

[0162] Generation of Plant Expression Vector pMON-PJU:

[0163] Briefly, the plant expression vector pMon316-PJU was constructed as follows. The isolated cDNA encoding *Arabidopsis* GPT (Example 1) was cloned into the ClaI-KpnI polylinker site of the pMON316 vector, which places the GPT gene under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) transcriptional terminator. A kanamycin resistance gene was included to provide a selectable marker.

Agrobacterium-Mediated Plant Transformations:

[0164] pMON-PJU and a control vector pMon316 (without inserted DNA) were transferred to *Agrobacterium tumefaciens* strain pTiTT37ASE using a standard electroporation method (McCormac et al., 1998, Molecular Biotechnology 9:155-159), followed by plating on LB plates containing the antibiotics spectinomycin (100 micro gm/ml) and kanamycin (50 micro gm/ml). Antibiotic resistant colonies of *Agrobacterium* were examined by PCR to assure that they contained plasmid.

[0165] *Nicotiana tabacum* cv. Xanthi plants were transformed with pMON-PJU transformed *Agrobacteria* using the leaf disc transformation system of Horsch et. al. (Horsch et al., 1995, Science 227:1229-1231). Briefly, sterile leaf disks were inoculated and cultured for 2 days, then transferred to selective MS media containing 100 μg/ml kanamycin and 500 μg/ml clafaran. Transformants were confirmed by their ability to form roots in the selective media.

Generation of GPT Transgenic Tobacco Plants:

[0166] Sterile leaf segments were allowed to develop callus on Murashige & Skoog (M&S) media from which the transformant plantlets emerged. These plantlets were then transferred to the rooting-permissive selection medium (M&S medium with kanamycin as the selection agent). The healthy, and now rooted, transformed tobacco plantlets were then transferred to soil and allowed to grow to maturity and upon flowering the plants were selfed and the resultant seeds were harvested. During the growth stage the plants had been examined for growth phenotype and the CO_2 fixation rate was measured for many of the young transgenic plants.

Production of T1 and T2 Generation GPT Transgenic Plants:

[0167] Seeds harvested form the T_0 generation of the transgenic tobacco plants were germinated on M&S media containing kanamycin (100 mg/L) to enrich for the transgene. At least one fourth of the seeds did not germinate on this media (kanamycin is expected to inhibit germination of the seeds without resistance that would have been produced as a result of normal genetic segregation of the gene) and more than half of the remaining seeds were removed because of demonstrated sensitivity (even mild) to the kanamycin.

[0168] The surviving plants (T_1 generation) were thriving and these plants were then selfed to produce seeds for the T_2 generation. Seeds from the T_1 generation were germinated on MS media supplemented for the transformant lines with kanamycin (10 mg/liter). After 14 days they were transferred to sand and provided quarter strength Hoagland's nutrient solution supplemented with 25 mM potassium nitrate. They were allowed to grow at 24° C. with a photoperiod of 16 h light and 8 hr dark with a light intensity of 900 micromoles per meter squared per second. They were harvested 14 days after being transferred to the sand culture.

Characterization of GPT Transgenic Plants:

[0169] Harvested transgenic plants (both GPT transgenes and vector control transgenes) were analyzed for glutamine sythetase activity in root and leaf, whole plant fresh weight, total protein in root and leaf, and CO_2 fixation rate (Knight et al., 1988, Plant Physiol. 88: 333). Non-transformed, wild-type *A. tumefaciens* plants were also analyzed across the same parameters in order to establish a baseline control.

[0170] Growth characteristic results are tabulated below in Table I. Additionally, a photograph of the GPT transgenic plant compared to a wild type control plant is shown in FIG. **2** (together with GS1 transgenic tobacco plant, see Example 5). Across all parameters evaluated, the GPT transgenic tobacco plants showed enhanced growth characteristics. In particular, the GPT transgenic plants exhibited a greater than 50% increase in the rate of CO_2 fixation, and a greater than two-fold increase in glutamine synthetase activity in leaf tissue, relative to wild type control plants. In addition, the leaf-to-root GS ratio increased by almost three-fold in the transaminase transgenic plants relative to wild type control. Fresh weight and total protein quantity also increased in the transgenic plants, by about 50% and 80% (leaf), respectively, relative to the wild type control. These data demonstrate that

tobacco plants overexpressing the *Arabidopsis* GPT transgene achieve significantly enhanced growth and CO_2 fixation rates.

TABLE I

	Leaf	Root
Protein mg/gram fresh weight		
Wild type - control	8.3	2.3
Line PN1-8 a second control	8.9	2.98
Line PN9-9	13.7	3.2
Glutamine Synthetase activity, micromoles/min/mg protein		
Wild type (Ratio of leaf:root = 4.1:1)	4.3	1.1
PN1-8 (Ratio of leaf:root = 4.2:1)	5.2	1.3
PN9-9 (Ratio of leaf:root = 10.9:1)	10.5	0.97
Whole Plant Fresh Weight, g		
Wild type	21.7	
PN1-8	26.1	
PN9-9	33.1	
CO_2 Fixation Rate, umole/m2/sec		
Wild type	8.4	
PN1-8	8.9	
PN9-9	12.9	

Data = average of three plants
Wild type - Control plants; not regenerated or transformed.
PN1 lines were produced by regeneration after transformation using a construct without inserted gene. A control against the processes of regeneration and transformation.
PN 9 lines were produced by regeneration after transformation using a construct with the Arabidopsis GPT gene.

Example 4

Generation of Transgenic Tomato Plants Carrying *Arabidopsis* GPT Transgene

[0171] Transgenic *Lycopersicon esculentum* (Micro-Tom Tomato) plants carrying the *Arabidopsis* GPT transgene were generated using the vectors and methods described in Example 3. T_0 transgenic tomato plants were generated and grown to maturity. Initial growth characteristic data of the GPT transgenic tomato plants is presented in Table II. The transgenic plants showed significant enhancement of growth rate, flowering, and seed yield in relation to wild type control plants. In addition, the transgenic plants developed multiple main stems, whereas wild type plants developed with a single main stem. A photograph of a GPT transgenic tomato plant compared to a wild type plant is presented in FIG. **3** (together with GS1 transgenic tomato plants, see Example 6).

TABLE II

Growth Characteristics	Wildtype Tomato	GPT Transgenic Tomato
Stem height, cm	6.5	18, 12, 11 major stems
Stems	1	3 major, 0 other
Buds	2	16
Flowers	8	12
Fruit	0	3

Example 5

Generation of Transgenic Tobacco Plants Overexpressing Alfalfa Gs1

[0172] Generation of Plant Expression Vector pGS111:

[0173] Transgenic tobacco plants overexpressing the Alfalfa GS1 gene were generated as previously described (Temple et al., 1993, Mol. Gen. Genetics 236: 315-325). Briefly, the plant expression vector pGS111 was constructed by inserting the entire coding sequence together with extensive regions of both the 5' and 3' untranslated regions of the Alfalfa GS1 gene [SEQ ID NO: 3] (DasSarma at al., 1986, Science, Vol 232, Issue 4755, 1242-1244) into pMON316 (Rogers et al., 1987, supra), placing the transgene under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) transcriptional terminator. A kanamycin resistance gene was included to provide a selectable marker.

Generation of GS1 Transformants:

[0174] pGS111 was transferred to *Agrobacterium tumefaciens* strain pTiTT37ASE using triparental mating as described (Rogers et al., 1987, supra; Unkefer et al., U.S. Pat. No. 6,555,500). *Nicotiana tabacum* cv. Xanthi plants were transformed with pGS111 transformed *Agrobacteria* using the leaf disc transformation system of Horsch et. al. (Horsch et al., 1995, Science 227:1229-1231). Transformants were selected and regenerated on MS medium containing 100 μg/ml kanamycin. Shoots were rooted on the same medium (with kanamycin, absent hormones) and transferred to potting soil:perlite:vermiculite (3:1:1), grown to maturity, and allowed to self. Seeds were harvested from this T_0 generation, and subsequent generations produced by selfing and continuing selection with kanamycin. The best growth performers were used to yield a T3 progeny for crossing with the best performing GPT over-expressing lines identified as described in Example 3. A photograph of the GS1 transgenic plant compared to a wild type control plant is shown in FIG. **2** (together with GPT transgenic tobacco plant, see Example 3)

Example 6

Generation of Transgenic Tomato Plants Carrying Alfalfa GS1 Transgene

[0175] Transgenic *Lycopersicon esculentum* (Micro-Tom Tomato) plants carrying the Alfalfa GS1 transgene were generated using the vector described in Example 5 and a transformation protocol essentially as described (Sun et al., 2006. Plant Cell Physiol. 46(3) 426-31). T_0 transgenic tomato plants were generated and grown to maturity. Initial growth characteristic data of the GPT transgenic tomato plants is presented in Table III. The transgenic plants showed significant enhancement of growth rate, flowering, and seed yield in relation to wild type control plants. In addition, the transgenic plants developed multiple main stems, whereas wild type plants developed with a single main stem. A photograph of a GS1 transgenic tomato plant compared to a wild type plant is presented in FIG. **3** (together with GPT transgenic tomato plant, see Example 4).

TABLE III

Growth Characteristics	Wildtype Tomato	GS1 Transgenic Tomato
Stem height, cm	6.5	16, 7, 5 major stems
Stems	1	3 major, 3 med, 1 sm
Buds	2	2
Flowers	8	13
Fruit	0	4

Example 7

Generation of Double Transgenic Tobacco Plants Carrying GS1 and GPT Transgenes

[0176] In an effort to determine whether the combination of GS1 and GPT transgenes in a single transgenic plant might improve the extent to which growth and other agronomic characteristics may be enhanced, a number of sexual crosses between high producing lines of the single transgene (GS1 or GPT) transgenic plants were carried out. The results obtained are dramatic, as these crosses repeatedly generated progeny plants having surprising and heretofore unknown increases in growth rates, biomass yield, and seed production.

Materials and Methods:

[0177] Single-transgene, transgenic tobacco plants overexpressing GPT or GS1 were generated as described in Examples 3 and 4, respectively. Several of fastest growing T_2 generation GPT transgenic plant lines were crossed with the fastest growing T3 generation GS1 transgenic plant lines using reciprocal crosses. The progeny were then selected on kanamycin containing M&S media as described in Example 3, and their growth, flowering and seed yields examined.

[0178] Tissue extractions for GPT and GS activities: GPT activity was extracted from fresh plant tissue after grinding in cold 100 mM Tris-HCl, pH 7.6, containing 1 mm ethylenediaminetetraacetic, 200 mM pyridoxal phosphate and 6 mM mercaptoethanol in a ratio of 3 ml per gram of tissue. The extract was clarified by centrifugation and used in the assay. GS activity was extracted from fresh plant tissue after grinding in cold 50 mM Imidazole, pH 7.5 containing 10 mM MgCl2, and 12.5 mM mercaptoethanol in a ratio of 3 ml per gram of tissue. The extract was clarified by centrifugation and used in the assay. GPT activity was assayed as described in Calderon and Mora, 1985, Journal Bacteriology 161:807-809. GS activity was measured as described in Shapiro and Stadtmann, 1970, Methods in Enzymology 17A: 910-922. Both assays involve an incubation with substrates and cofactor at the proper pH. Detection was by HPLC.

Results:

[0179] The results are presented in two ways. First, specific growth characteristics are tabulated in Tables IV.A and IV.B (biomass, seed yields, growth rate, GS activity, GPT activity, 2-oxoglutaramate activity, etc). Second, photographs of progeny plants and their leaves are shown in comparison to single-transgene and wild type plants and leaves are presented in FIG. **5** and FIG. **6**, which show much larger whole plants, larger leaves, and earlier and/or more abundant flowering in comparison to the parental single-transgene plants and wild type control plants.

[0180] Referring to Table IV.A, double-transgene progeny plants form these crosses showed tremendous increases total biomass (fresh weight), with fresh weights ranging from 45-89 grams per individual progeny plant, compared to a range of only 19-24 grams per individual wild type plant, representing on average, about a two- to three-fold increase over wild type plants, and representing at the high end, an astounding four-fold increase in biomass over wild type plants. Taking the 24 individual double-transgene progeny plants evaluated, the average individual plant biomass was about 2.75 times that of the average wild type control plant. Four of the progeny lines showed approximately 2.5 fold greater average per plant fresh weights, while two lines showed over three-fold greater fresh weights in comparison to wild type plants.

[0181] In comparison to the single-transgene parental lines, the double-transgene progeny plants also showed far more than an additive growth enhancement. Whereas GPT single-transgene lines show as much as about a 50% increase over wild type biomass, and GS1 single-transgene lines as much as a 66% increase, progeny plants averaged almost a 200% increase over wild type plants.

[0182] Similarly, the double transgene progeny plants flowered earlier and more prolifically than either the wild type or single transgene parental lines, and produced a far greater number of seed pods as well as total number of seeds per plant. Referring again to Table IV.A, on average, the double-transgene progeny produced over twice the number of seed pods produced by wild type plants, with two of the high producer plants generating over three times the number of seed pods compared to wild type. Total seed yield in progeny plants, measured on a per plant weight basis, ranged from about double to nearly quadruple the number produced in wild type plants.

TABLE IV.A

PLANT LINE	FRESH WEIGHT g/whole plant	SEED PODS #pods/plant	SEED YIELD g/plant	GS ACTIVITY LEAF	GS ACTIVITY ROOT	GS ACTIVITY L/R RATIO
Wild Type Tobacco						
Wild type 1	18.73	26	0.967			
Wild type 2	24.33	24	1.07			
Wild type 3	23.6	32	0.9			
Wild type 4	18.95	32	1.125			
WT Average	21.4025	28.5	1.0155	7.75	1.45	5.34
Cross 1 X1L1a × PA9-9ff						
1	59.21	62	2.7811			
2	65.71	56				
3	55.36	72				
4	46.8	56				

TABLE IV.A-continued

PLANT LINE	FRESH WEIGHT g/whole plant	SEED PODS #pods/plant	SEED YIELD g/plant	GS ACTIVITY LEAF	GS ACTIVITY ROOT	L/R RATIO
Cross 1 Average	56.77	61.5	+274%	14.98	1.05	14.27
Compared to WT	+265%	+216%		+193%	−28%	+267%
Cross 2						
PA9-2 × L9						
1	70.83	61	1.76			
2	49.17	58	3.12			
3	50.23	90 NA				
4	45.77					
Cross 2 Average	54	58.3	2.44	16.32	1.81	9.02
Compared to WT	+252%	+205%	+240%	+211%	+125%	+169%
Cross 3						
PA9-9ff × L1a						
1	89.1	77	3.687			
2	78.18					
3	58.34					
4	61.79					
Cross 3 Average	71.85	77 (one plant)	3.678 (one plant)	15.92	1.38	11.54
Compared to WT	+336%	+270%	+362%	+205%	−5%	+216%
Cross 5						
PA9-10aa × L1a						
1	65.34	45	2.947			
2	53.28	64	3.3314			
3	49.85	42	1.5667			
4	44.63	42	2.5013			
Cross 5 Average	53.275	48.25	2.86928	13.03	1.8	7.24
Compared to WT	+244%	+169%	+283%	+168%		
Cross 6						
PA9-17b × L1a						
1	56.7	64	2.492			
2	55.05	66	2.162			
3	51.51	59	1.8572			
4	45.38	72	4.742			
Cross 6 Average	52.16	65.25	2.8133	14.114.752	1.1.1124	13.29
Compared to WT	+244%	+229%	+277%			
Cross 7						
PA9-20aa × L1b						
1	76.26	67	2.0535			
2	66.27	42	1.505			
3	72.26	72	2.3914			
4	63.91	91	2.87			
Cross 7 Average	69.675	68	2.204975	14.12	1.24	11.39
Compared to WT	+326%	+239%	+217%			
Control PA9-9ff						
1	32.18	N/A				
2	32.64	N/A				
3	34.67	N/A				
4	25.18	N/A				
Average	31.17	N/A		11.57	1.14	10.15
Compared to WT	+148%					
Control GS L1a						
1	41.74	N/A				
2	36.24	N/A				
3	33.8	N/A				
4	30.48	N/A				
Average	35.57	N/A		13.15	1.23	10.69
Compared to WT	+166%					

[0183] Table IV.B shows growth rate, biomass and yield, and biochemical characteristics of Line XX (Line 3 further selfed) compared to the single transgene line expressing GS1 and wild type control tobacco. All parameters are greatly increased in the double transgenic plant (Line XX). Notably, 2-oxoglutaramate activity was almost 17-fold higher, and seed yield and foliar biomass was three-fold higher, in Line XX plants versus control plants.

TABLE IV.B

Plant Type	Specific Growth Rate mg/g/d	Foliar Biomass FWt, g	Fruit/ Flowers/ Buds	Seed Yield g	GS Activity umol/min/ gFWt	GPT Activity nmol/h/ gFWt	2-oxoglu-taramate nmol/gFWt	Trans Gene Assay
Wildtype, avg	228	21.40	28.5	1.02	7.75	16.9	68.9	No
Line 1 GS	269	35.57	NM	NM	11.6	NM	414	Yes
Line XX	339	59.71	62.9	2.94	16.3	243.9	1,153.6	Yes

NM Not Measured

Example 8

Generation of Double Transgenic Pepper Plants Carrying GS1 and GPT Transgenes

[0184] In this example, Big Jim chili pepper plants (New Mexico varietal) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter, and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter, using *Agrobacterium*-mediated transfer to seed pods. After 3 days, seeds were harvested and used to generate T0 plants and screened for transformants. The resulting double-transgenic plants showed higher pod yields, faster growth rates, and greater biomass yields in comparison to the control plants.

Materials and Methods:

[0185] *Solanaceae Capisicum* Pepper plants ("Big Jim" varietal) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON (see Example 3), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (Tomato rubisco rbcS3C promoter: Kyozulka et al., 1993, Plant Physiol. 103: 991-1000; SEQ ID NO: 22; vector construct of SEQ ID NO: 6), using *Agrobacterium*-mediated transfer to seed pods.

[0186] For this and all subsequent examples, the Cambia 1201 or 1305.1 vectors were constructed according to standard cloning methods (Sambrook et al., 1989, supra, Saiki et al., 1988, Science 239: 487-491). The vector is supplied with a 35S CaMV promoter; that promoter was replaced with RcbS-3C promoter from tomato to control the expression of the target gene. The Cambia 1201 vectors contain bacterial chlorophenicol and plant hygromycin resistance selectable marker genes. The Cambia 1305.1 vectors contain bacterial chlorophenicol and hygromycin resistance selectable marker genes.

[0187] The transgene expression vectors pMON (GPT transgene) and pCambia 1201 (GS transgene) were transferred to separate *Agrobacterium tumefaciens* strain LBA4404 cultures using a standard electroporation method (McCormac et al., 1998, Molecular Biotechnology 9:155-159). Transformed *Agrobacterium* were selected on media containing 50 μg/ml of either streptamycin for pMON constructs or chloroamphenicol for the Cambia constructs. Transformed *Agrobacterium* cells were grown in LB culture media containing 25 μg/ml of antibiotic for 36 hours. At the end of the 36 hr growth period cells were collected by centrifugation and cells from each transformation were resuspended in 100 ml LB broth without antibiotic.

[0188] Pepper plants were then transformed with a mixture of the resulting *Agrobacterium* cell suspensions using a transformation protocol in which the *Agrobacterium* is injected directly into the seed cavity of developing pods. Briefly, developing pods were injected with the 200 ml mixture in order to inoculate immature seeds with the *Agrobacteria* essentially as described (Wang and Waterhouse, 1997, Plant Mol. Biol. Reporter 15: 209-215). In order to induce *Agrobacteria* virulence and improve transformation efficiencies, 10 μg/ml acetosyringonone was added to the *Agrobacteria* cultures prior to pod inoculations (see, Sheikholeslam and Weeks, 1986, Plant Mol. Biol. 8: 291-298).

[0189] Using a syringe, pods were injected with a liberal quantity of the *Agrobacterium* vector mixture, and left to incubate for about 3 days. Seeds were then harvested and germinated, and developing plants observed for phenotypic characteristics including growth and antibiotic resistance. Plants carrying the transgenes were green, whereas untransformed plants showed signs of chlorosis in leaf tips. Vigorous growing transformants were further cultivated and compared to wild type pepper plants grown under identical conditions.

Results:

[0190] The results are presented in FIG. **7** and Table V. FIG. **7** shows a photograph of a GPT+GS double transgenic pepper plant compared to a control plant grown for the same time under identical conditions. This photograph shows tremendous pepper yield in the transgenic line compared to the control plant.

[0191] Table V presents biomass yield and GS activity, as well as transgene genotyping, in the transgenic lines compared to the wild type control. Referring to Table V, double-transgene progeny plants showed tremendous increases total biomass (fresh weight), with fresh weights, ranging from 393-662 grams per individual transgenic plant, compared to an average of 328 grams per wild type plant. Transgenic line A5 produced more than twice the total biomass of the controls. Moreover, pepper yields in the transgenic lines were greatly improved over wild type plants, and were 50% greater

than control plants (on average). Notably, one of the transgene lines produced twice as many peppers as the control plant average.

TABLE V

TRANSGENIC PEPPER GROWTH/BIOMASS AND REPRODUCTION

Plant type	Biomass, Foliar Fresh Wt, g	Yield Peppers, g DWt	GS activity Umoles/min/ gFWt	Transgene Presence Assay
Wildtype, avg	328.2	83.7	1.09	Negative
Line A2	457.3	184.2	1.57	GPT - Yes
Line A5	661.7	148.1	1.8	GPT - Yes
Line B1	493.4	141.0	1.3	GPT - Yes
Line B4	393.1	136.0	1.6	GPT - Yes
Line C1	509.4	152.9	1.55	GPT - Yes

FWt Fresh Weight;
DWt Dry Weight

Example 9

Generation of Double Transgenic Bean Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0192] In this example, yellow wax bean plants (*Phaseolus vulgaris*) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pCambia 1201, and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201, using *Agrobacterium*-mediated transfer into flowers.

Materials and Methods:

[0193] The transgene expression vectors pCambia 1201-GPT (including construct of SEQ ID NO: 27) and pCambia 1201-GS (including construct of SEQ ID NO: 6) were transferred to separate *Agrobacterium tumefaciens* strain LBA4404 cultures using a standard electroporation method (McCormac et al., 1998, Molecular Biotechnology 9:155-159). Transformed *Agrobacterium* were selected on media containing 50 μg/ml of chloroamphenicol. Transformed *Agrobacterium* cells were grown in LB culture media containing 25 μg/ml of antibiotic for 36 hours. At the end of the 36 hr growth period cells were collected by centrifugation and cells from each transformation were resuspended in 100 ml LB broth without antibiotic.

[0194] Bean plants were then transformed with a mixture of the resulting *Agrobacterium* cell suspensions using a transformation protocol in which the *Agrobacteria* is injected directly into the flower structure (Yasseem, 2009, Plant Mol. Biol. Reporter 27: 20-28). In order to induce *Agrobacteria* virulence and improve transformation efficiencies, 10 μg/ml acetosyringonone was added to the *Agrobacteria* cultures prior to flower inoculation. Briefly, once flowers bloomed, the outer structure encapsulating the reproductive organs was gently opened with forceps in order to permit the introduction of the *Agrobacteria* mixture, which was added to the flower structure sufficient to flood the anthers.

[0195] Plants were grown until bean pods developed, and seeds were harvested and used to generate transgenic plants. Transgenic plants were then grown together with control bean plants under identical conditions, photographed and phenotypically characterized. Growth rates were measured for both transgenic and control plants. In this and all examples, Glutamine synthetase (GS) activity was assayed according to the methods in Shapiro and Stadtmann, 1970, Methods in Enzymology 17A: 910-922; and, Glutamine phenylpyruvate transaminase (GPT) activity was assayed according to the methods in Calderon et al., 1985, J. Bacteriol. 161: 807-809. See details in Example 7, Methods, supra.

Results:

[0196] The results are presented in FIG. **8**, FIG. **9** and Table VI.

[0197] FIG. **8** shows GPT+GS transgenic bean line A growth rate data relative to control plants, including plant heights on various days into cultivation, as well as numbers of flower buds, flowers, and bean pods. These data show that the GPT+GS double transgenic bean plants outgrew their counterpart control plants. The transgenic plants grew taller, flowered earlier and produced more flower buds and flowers, and developed bean pods and produced more bean pods that the wild type control plants.

TABLE VI

TRANSGENIC BEANS LINE A

Plant Type	Bean Pod Yield FWt, g	GPT Activity nmoles/h/gFWt	GS Activity umoles/min/ gFWt	Antibiotic Resistance
Wildtype, avg	126.6	101.9	25.2	Negative
2A	211.5	NM	NM	+
4A	207.7	NM	NM	+
5B	205.7	984.7	101.3	+

WT Wildtype;
FWt Fresh Weight;
NM Not Measured

[0198] Table VI presents bean pod yield, GPT and GS activity, as well as antibiotic resistance status, in the transgenic lines compared to the wild type control (average of several robust control plants; control plants that did not grow well were excluded from the analyses). Referring to Table VI, double-transgene progeny plants showed substantial bean pod biomass increases (fresh pod weight) in comparison to the control plants, with bean pod biomass yields consistently above 200 grams per individual transgenic plant, compared to an average of 127 grams per wild type plant, representing an over 60% increase in pod yield in the double transgene lines relative to control plant(s).

[0199] Lastly, FIG. **9** shows a photograph of a GPT+GS double transgenic bean plant compared to a control plant grown for the same time under identical conditions, showing increased growth in the transgenic plant.

Example 10

Generation of Double Transgenic Bean Plants Carrying *Arabidopsis* GS1 and Grape GPT Transgenes

[0200] In this example, yellow wax bean plants (*Phaseolus vulgaris*) were transformed with the Grape GPT full length coding sequence included in SEQ ID NO: 8 under the control of the RuBisCo promoter within the expression vector pCambia 1305.1, and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo

promoter within the expression vector pCambia 1201, using *Agrobacterium*-mediated transfer into developing pods.

Materials and Methods:

[0201] The transgene expression vectors pCambia 1201-GPT(grape) (including construct of SEQ ID NO: 8) and pCambia 1201-GS (including construct of SEQ ID NO: 6) were transferred to separate *Agrobacterium tumefaciens* strain LBA4404 cultures using a standard electroporation method (McCormac et al., 1998, Molecular Biotechnology 9:155-159). Transformed *Agrobacterium* were selected on media containing 50 μg/ml of chloroamphenicol. Transformed *Agrobacterium* cells were grown in LB culture media containing 25 μg/ml of antibiotic for 36 hours. At the end of the 36 hr growth period cells were collected by centrifugation and cells from each transformation were resuspended in 100 ml LB broth without antibiotic.

[0202] Bean plants were then transformed with a mixture of the resulting *Agrobacterium* cell suspensions using a transformation protocol in which the *Agrobacteria* is injected directly into the flower structure. In order to induce *Agrobacteria* virulence and improve transformation efficiencies, 10 μg/ml acetosyringonone was added to the *Agrobacteria* cultures prior to flower inoculation. Briefly, once flowers bloomed, the outer structure encapsulating the reproductive organs was gently opened with forceps in order to permit the introduction of the *Agrobacteria* mixture, which was added to the flower structure sufficient to flood the anthers.

[0203] Plants were grown until bean pods developed, and seeds were harvested and used to generate transgenic plants. Transgenic plants were then grown together with control bean plants under identical conditions, photographed and phenotypically characterized. Growth rates were measured for both transgenic and control plants.

Results:

[0204] The results are presented in FIG. **10**, FIG. **11** and Table VII.

[0205] FIG. **10** shows GPT+GS transgenic bean line G growth rate data relative to control plants, specifically including numbers of flower buds, flowers, and bean pods. These data show that the GPT+GS double transgenic bean plants outgrew their counterpart control plants. Notably, the transgenic plants produced substantially more bean pods that the wild type control plants.

TABLE VII

TRANSGENIC BEANS LINE G: POD YIELDS

Plant Type	Bean Pod Yield FWt, g	Antibiotic Resistance
Wild type, avg	157.9	Negative
G1	200.5	+
G2	178.3	+

WT Wildtype;
FWt Fresh Weight;
NM Not Measured

[0206] Table VII presents bean pod yield and antibiotic resistance status, in the transgenic lines compared to the wild type control (average of several robust control plants; control plants that did not grow well were excluded from the analyses). Referring to Table VII, double-transgene progeny plants showed substantial bean pod biomass increases (fresh pod weight) in comparison to the control plants, with bean pod biomass yields of 200.5 (line G1) and 178 grams (line G2) per individual transgenic plant, compared to an average of 158 grams per individual wild type plant, representing approximately a 27% increase in pod yield in the double transgene lines relative to control plants.

[0207] Lastly, FIG. **11** shows a photograph of a GPT+GS double transgenic bean plant compared to a control plant grown for the same time under identical conditions. The transgenic plant shows substantially increased size and biomass, larger leaves and a more mature flowering compared to the control plant.

Example 11

Generation of Double Transgenic Cowpea Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0208] In this example, common Cowpea plants were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON, and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201, using *Agrobacterium*-mediated transfer into flowers. Materials and methods were as in Example 9, supra.

Results:

[0209] The results are presented in FIGS. **12** and **13**, and Table VI. FIG. **12** shows relative growth rates for the GPT+GS transgenic Cowpea line A and wild type control Cowpea at several intervals during cultivation, including (FIG. **12**A) height and longest leaf measurements, (FIG. **12**B) trifolate leafs and flower buds, and (FIG. **12**C) flowers, flower buds and pea pods. These data show that the GPT+GS double transgenic Cowpea plants outgrew their counterpart control plants. The transgenic plants grew faster and taller, had longer leaves, and set flowers and pods sooner than wild type control plants.

TABLE VIII

TRANSGENIC COWPEA LINE A

Plant Type	Pea Pod Yield, FWt, g	GPT Activity nmoles/h/gFWt	GS Activity umol/min/gFWt	Antibiotic Resistance
Wildtype, avg	74.7	44.4	28.3	Negative
4A	112.8	NM	41.3	+
8B	113.8	736.2	54.9	+

WT Wildtype;
FWt Fresh Weight;
NM Not Measured

[0210] Table VIII presents pea pod yield, GPT and GS activity, as well as antibiotic resistance status, in the transgenic lines compared to the wild type control (average of several robust control plants; control plants that did not grow well were excluded from the analyses). Referring to Table VIII, double-transgene progeny plants showed substantial pea pod biomass increases (fresh pod weight) in comparison to the control plants, with average transgenic plant pea pod biomass yields nearly 52% greater than the yields measured in control plant(s).

[0211] Lastly, FIG. **13** shows a photograph of a GPT+GS double transgenic bean plant compared to a control plant grown for the same time under identical conditions, showing increased biomass and pod yield in the transgenic plant relative to the wild type control plant.

Example 12

Generation of Double Transgenic Cowpea Plants Carrying *Arabidopsis* GS1 and Grape GPT Transgenes

[0212] In this example, common Cowpea plants were transformed with the Grape GPT full length coding sequence included in SEQ ID NO: 8 under the control of the RuBisCo promoter within the expression vector pCambia 1305.1 (including construct of SEQ ID NO: 8), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6), using *Agrobacterium*-mediated transfer into flowers. Materials and methods were as in Example 11, supra.

Results:

[0213] The results are presented in FIGS. **14** and **15**, and Table IX.

[0214] FIG. **14** shows relative growth rates for the GPT+GS transgenic Cowpea line G and wild type control Cowpea. These data show that the transgenic plants are consistently higher (FIG. **14**A), produce substantially more flowers, flower buds and pea pods (FIG. **14**B), and develop trifolates and leaf buds faster (FIG. **14**C).

TABLE IX

TRANSGENIC COWPEA LINE G

Plant Type	Pod Yield, FWt, g	GPT Activity nmoles/h/gFWT	GS Activity umol/min/gFWt	Antibiotic Resistance
Wildtype, avg	59.7	44.4	26.7	Negative
G9	102.0	555.6	34.5	+

WT Wildtype;
FWt Fresh Weight;
NM Not Measured

[0215] Table IX presents pea pod yield, GPT and GS activity, as well as antibiotic resistance status, in the transgenic lines compared to the wild type control (average of several robust control plants; control plants that did not grow well were excluded from the analyses). Referring to Table IX, double-transgene progeny plants showed substantial pea pod biomass increases (fresh pod weight) in comparison to the control plants, with average pea pod biomass yields 70% greater in the transgenic plants compared to control plant(s).

[0216] Lastly, FIG. **15** shows a photograph of a GPT+GS double transgenic pea plant compared to a control plant grown for the same time under identical conditions, showing increased height, biomass and leaf size in the transgenic plant relative to the wild type control plant.

Example 13

Generation of Double Transgenic Alfalfa Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0217] In this example, Alfalfa plants (*Medicago sativa*, var *Ladak*) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON316 (see Example 3, supra), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6), using *Agrobacterium*-mediated transfer into seedling plants. *Agrobacterium* vectors and mixtures were prepared for seedling inoculations as described in Example 11, supra.

Seedling Inoculations:

[0218] When Alfalfa seedlings were still less than about ½ inch tall, they were soaked in paper toweling that had been flooded with the *Agrobacteria* mixture containing both transgene constructs. The seedlings were left in the paper toweling for two to three days, removed and then planted in potting soil. Resulting T0 and control plants were then grown for the first 30 days in a growth chamber, thereafter cultivated in a greenhouse, and then harvested 42 days after sprouting. At this point, only the transgenic Alfalfa line displayed flowers, as the wild type plants only displayed immature flower buds. The plants were characterized as to flowering status and total biomass.

Results:

[0219] The results are presented in Table X. The data shows that the transgenic Alfalfa plants grew faster, flowered sooner, and yielded on average about a 62% biomass increase relative to the control plants.

TABLE X

TRANSGENIC ALFALFA VS. CONTROL

Plant Type	Biomass at Sacrifice, g	Flowering Stage
Wildtype, avg	6.03	Small defined buds No buds swelling. No flowers
Transgene #5	10.38	4 Open flowers
Transgene # 11	9.03	Flower buds swelling
Transgene #13	9.95	Flower buds swelling

Example 14

Generation of Double Transgenic Cantaloupe Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0220] In this example, Cantaloupe plants (*Cucumis melo* var common) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON316 (see Example 3, supra), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6), using *Agrobacterium*-mediated transfer via injection into developing melons. *Agrobacterium* vectors and mixtures were prepared for intra-melon inoculations as described in Example 8, supra. Inoculations into developing melons were carried out essentially as described in

Example 8

[0221] The plants were characterized as to flowering status and total biomass relative to control melon plants grown under identical conditions.

[0222] The results are presented in FIG. **16** and Table XI. Referring to Table XI, the transgenic plants showed substantial foliar plant biomass increases in comparison to the control plants, with an average increase in biomass of 63%. Moreover, a tremendous increase in flower and flower bud yields was observed in all five transgenic lines. Control plants displayed no flowers and only 5 buds at sacrifice, on average. In sharp contrast, the transgenic plants displayed between 2 and 5 flowers per plant, and between 21 and 30 flower buds, per plant, indicating a substantially higher growth rate and flower yield. Increased flower yield would be expected to translate into correspondingly higher melon yields in the transgenic plants. Referring to FIG. **16** (a photograph comparing transgenic Cantaloupe plants to control Cantaloupe plants), the transgenic Cantaloupe plants show dramatically increased height, overall biomass and flowering status relative to the control plants.

TABLE XI

TRANGENIC CANTALOUPE VERSUS CONTROL

Plant Type	Biomass Foliar FWt, g	Flowers/Flower Buds at Sacrifice	Antibiotic Resistance
Wildtype, avg	22.8	0/5	Negative
Line 1	37.0	3/21	+
Line 2	35.0	2/30	+
Line 3	37.1	3/27	+
Line 4	40.6	5/26	+
Line 5	35.7	4/30	+

FWt Fresh Weight

Example 15

Generation of Double Transgenic Pumpkin Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0223] In this example, common Pumpkin plants (*Cucurbita maxima*) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON316 (see Example 3, supra), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6), using *Agrobacterium*-mediated transfer via injection into developing pumpkins, essentially as described in Example 14, supra. The transgenic and control pumpkin plants were grown under identical conditions until the emergence of flower buds in the control plants, then all plants were characterized as to flowering status and total biomass.

[0224] The results are presented in FIG. **17** and Table XII. Referring to Table XII, the transgenic plants showed substantial foliar plant biomass increases in comparison to the control plants, with an increase in average biomass yield of 67% over control plants. Moreover, an increase in flower bud yields was observed in four of the five transgenic lines in comparison to control. Control plants displayed only 4 buds at sacrifice (average). In contrast, four transgenic plant lines displayed between 8 and 15 flowers buds per plant, representing a two- to nearly four-fold yield increase.

TABLE XII

TRANGENIC PUMPKIN VERSUS CONTROL

Plant Type	Biomass Foliar FWt, g	Flower Buds at Sacrifice	Antibiotic Resistance
Wildtype, avg	47.7	4.2	Negative
Line 1 (Photo)	82.3	8	
Line 2	74.3	8	+
Line 3	80.3	9	+
Line 4 (Photo)	77.8	4	+
Line 5	84.5	15	+

FWt Fresh Weight;

[0225] Referring to FIG. **17** (a photograph comparing transgenic pumpkin plants to control plants), the transgenic pumpkin plants show substantially increased plant size, overall biomass and leaf sizes and numbers relative to the control plants.

Example 16

Generation of Double Transgenic *Arabidopsis* Plants Carrying *Arabidopsis* GS1 and GPT Transgenes

[0226] In this example, *Arabidopsis thaliana* plants were transformed with the truncated *Arabidopsis* GPT coding sequence of SEQ ID NO: 18 under the control of the CMV 35S promoter within the expression vector pMON316 (see Example 3, supra), and transgenic plants thereafter transformed with the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6), using *Agrobacterium*-mediated "floral dip" transfer as described (Harrison et al., 2006, Plant Methods 2:19-23; Clough and Bent, 1998, Plant J. 16:735-743). *Agrobacterium* vectors pMON316 carrying GPT and pCambia 1201 carrying GS1 were prepared as described in Examples 3 and 11, respectively.

[0227] Transformation of two different cultures of *Agrobacterium* with either a pMon 316+ *Arabidopsis* GTP construct or with a Cambia 1201+ *Arabidopsis* GS construct was done by electroporation using the method of Weigel and Glazebrook 2002. The transformed *Agrobacterium* were then grown under antibiotic selection, collected by centrifugation resuspended in LB broth with antibiotic and used in the floral dip of *Arabidopsis* inflorescence. Floral dipped *Arabidopsis* plants were taken to maturity and self-fertilized and seeds were collected. Seeds from twice dipped plants were first geminated on a media containing 20 ug/ml of kanamycin and by following regular selection procedures surviving seedlings were transferred to media containing 20 ug of hygromycin. Plants (3) surviving the selection process on both antibiotics were self-fertilized and seeds were collected. Seeds from the T1 generation were germinated on MS media containing 20 ug/ml of hygromycin and surviving seedlings were taken to maturity, self-fertilized and seeds collected. This seed population the T2 generation was then used for subsequent growth studies.

[0228] The results are presented in FIG. **18** and Table XIII. Referring to Table XIII, which shows data from 6 wild type and 6 transgenic *Arabidopsis* plants (averaged), the transgenic plants displayed increased levels of both GPT and GS activity. GPT activity was over twenty-fold higher than the control plants. Moreover, the transgenic plant fresh foliar

weight average was well over four-fold that of the wild type control plant average. A photograph of young transgene *Arabidopsis* plants in comparison to wild type control *Arabidopsis* plants grown under identical conditions is shown in FIG. **18**, and reveals a consistent and very significant growth/biomass increase in transgenic plants relative to the control plants.

TABLE XIII

TRANSGENIC ARABIDOPSIS VERSUS CONTROL

Plant type	Biomass, g Fresh foliar wt	GPT Activity nmol/h/gFWt	GS Activity umol/min/gFWt	Antibiotic Resistance
Wildtype, avg	0.246	18.4	7.0	Negative
Transgene	1.106	395.6	18.2	Positive

Example 17

Generation of Transgenic Tomato Plants Carrying *Arabidopsis* GPT and GS1 Transgenes

[0229] In this example, tomato plants (*Solanum lycopersicon*, "Money Maker" variety) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the CMV 35S promoter within the expression vector pMON316 (see Example 3, supra), and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201 (including construct of SEQ ID NO: 6). Single transgene (GPT) transgenic tomato plants were generated and grown to flowering essentially as described in Example 4. The *Arabidopsis* GS1 transgene was then introduced into the single-transgene T0 plants using *Agrobacterium*-mediated transfer via injection directly into flowers (as described in Example 8). The transgenic and control tomato plants were grown under identical conditions and characterized as to growth phenotype characteristics. Resulting T0 double-transgene plants were then grown to maturity, photographed along with control tomato plants, and phenotypically characterized.

[0230] The results are presented in FIG. **19** and in Table X. Referring to Table XIX, double-transgene tomato plants showed substantial foliar plant biomass increases in comparison to the control plants, with an increase in average biomass yield of 45% over control. Moreover, as much as a 70% increase in tomato fruit yield was observed in the transgenic lines compared to control plants (e.g., 51 tomatoes harvested from Line 4C, versus and average of approximately 30 tomatoes from control plants). A much higher level of GPT activity was observed in the transgenic plants (e.g., line 4C displaying an approximately 32-fold higher GPT activity in comparison to the average GPT activity measured in control plants). GS activity was also higher in the transgenic plants relative to control plants (almost double in Line 4C).

[0231] With respect to growth phenotype, and referring to FIG. **19**, the transgenic tomato plants displayed substantially larger leaves compared to control plants (FIG. **19**A). In addition, it can be seen that the transgenic tomato plants were substantially larger, taller and of a greater overall biomass (see FIG. **19**B).

TABLE XIX

TRANSGENIC TOMATO GROWTH AND REPRODUCTION

Plant Type	Biomass Foliar FWt, g	Total Tomatoes Harvested until Sacrifice	GPT Activity nmoles/h/gFWt	GS Activity umoles/min/gFWt	Transgene Presence Assay
Wildtype, avg	891	30.2	287	14.27	Negative
Line 6C	1288	43	9181	18.3	+
Line 4C	1146	51	1718	26.4	+

Example 18

Generation of Transgenic Camilena Plants Carrying *Arabidopsis* GPT and GS1 Transgenes

[0232] In this example, Camelina plants (*Camelina sativa*, Var MT 303) were transformed with the *Arabidopsis* GPT full length coding sequence of SEQ ID NO: 1 under the control of the RuBisCo promoter within the expression vector pCambia 1201, and the *Arabidopsis* GS1 coding sequence included in SEQ ID NO: 6 under the control of the RuBisCo promoter within the expression vector pCambia 1201, using *Agrobacterium*-mediated transfer into germinating seeds according to the method described in Chee et al., 1989, Plant Physiol. 91: 1212-1218. *Agrobacterium* vectors and mixtures were prepared for seed inoculations as described in Example 11, supra.

[0233] Transgenic and control Camelina plants were grown under identical conditions (30 days in a growth chamber and then moved to greenhouse cultivation) for 39 days, and characterized as to biomass, growth characteristics and flowering stage.

[0234] The results are presented in Table XX and FIG. **20**. Referring to Table XX, it can be seen that total biomass in the transgenic plants was, on average, almost double control plant biomass. Canopy diameter was also significantly improved in the transgenic plants. FIG. **20** shows a photograph of transgenic Camelina compared to control. The transgenic plant is noticeably larger and displays more advanced flowering.

TABLE XX

TRANSGENIC CAMELINA VERSUS CONTROL

Plant Type	Height/Canopy Diameter, inches	Biomass G	Flowering Stage
Wildtype, avg	14/4	8.35	Partial flowering
Transgene C-1	15.5/5	16.54	Full flowering
Transgene C-3	14/7	14.80	Initial flowering

Example 19

Activity of Barley GPT Transgene in Planta

[0235] In this example, the putative coding sequence for Barley GPT was isolated and expressed from a transgene construct using an in planta transient expression assay. Biologically active recombinant Barley GPT was produced, and catalyzed the increased synthesis of 2-oxoglutaramate, as confirmed by HPLC.

[0236] The Barley (*Hordeum vulgare*) GPT coding sequence was determined and synthesized. The DNA sequence of the Barley GPT coding sequence used in this example is provided in SEQ ID NO: 14, and the encoded GPT protein amino acid sequence is presented in SEQ ID NO: 15.

[0237] The coding sequence for Barley GPT was inserted into the 1305.1 cambia vector, and transferred to *Agrobacterium tumefaciens* strain LBA404 using a standard electroporation method (McCormac et al., 1998, Molecular Biotechnology 9:155-159), followed by plating on LB plates containing hygromycin (50 micro gm/ml). Antibiotic resistant colonies of *Agrobacterium* were selected for analysis.

[0238] The transient tobacco leaf expression assay consisted of injecting a suspension of transformed *Agrobacterium* (1.5-2.0 OD 650) into rapidly growing tobacco leaves. Intradermal injections were made in a grid across the leaf surface to assure that a significant amount of the leaf surface would be exposed to the *Agrobacterium*. The plant was then allowed to grow for 3-5 days when the tissue was extracted as described for all other tissue extractions and the GPT activity measured.

[0239] GPT activity in the inoculated leaf tissue (1217 nanomoles/gFWt/h) was three-fold the level measured in the control plant leaf tissue (407 nanomoles/gFWt/h), indicating that the *Hordeum* GPT construct directed the expression of biologically active GPT in a transgenic plant.

Example 20

Isolation and Expression of Recombinant Rice GPT Gene Coding Sequence and Analysis of Biological Activity

[0240] In this example, the putative coding sequence for rice GPT was isolated and expressed in *E. coli*. Biologically active recombinant rice GPT was produced, and catalyzed the increased synthesis of 2-oxoglutaramate, as confirmed by HPLC.

Materials and Methods:

[0241] Rice GPT Coding Sequence and Expression in *E. coli:*

[0242] The rice (*Oryza sativa*) GPT coding sequence was determined and synthesized, inserted into a PET28 vector, and expressed in *E. coli*. Briefly, *E. coli* cells were transformed with the expression vector and transformants grown overnight in LB broth diluted and grown to OD 0.4, expression induced with isopropyl-B-D-thiogalactoside (0.4 micromolar), grown for 3 hr and harvested. A total of 25×106 cells were then assayed for biological activity using the NMR assay, below. Untransformed, wild type *E. coli* cells were assayed as a control. An additional control used *E. coli* cells transformed with an empty vector.

[0243] The DNA sequence of the rice GPT coding sequence used in this example is provided in SEQ ID NO: 10, and the encoded GPT protein amino acid sequence is presented in SEQ ID NO: 11.

HPLC Assay for 2-Oxoglutaramate:

[0244] HPLC was used to determine 2-oxoglutaramate production in GPT-overexpressing *E. coli* cells, following a modification of Calderon et al., 1985, J Bacteriol 161(2): 807-809. Briefly, a modified extraction buffer consisting of 25 mM Tris-HCl pH 8.5, 1 mM EDTA, 20 μM Pyridoxal phosphate, 10 mM Cysteine, and ~1.5% (v/v) Mercaptoethanol was used. Samples (lysate from *E. coli* cells, 25×106 cells) were added to the extraction buffer at approximately a 1/3 ratio (w/v), incubated for 30 minutes at 37° C., and stopped with 200 μl of 20% TCA. After about 5 minutes, the assay mixture is centrifuged and the supernatant used to quantify 2-oxoglutaramate by HPLC, using an ION-300 7.8 mm ID×30 cm L column, with a mobile phase in 0.01N H_2SO_4, a flow rate of approximately 0.2 ml/min, at 40° C. Injection volume is approximately 20 and retention time between about 38 and 39 minutes. Detection is achieved with 210 nm UV light.

[0245] NMR analysis comparison with authentic 2-oxoglutaramate was used to establish that the Arabidopsis full length sequence expresses a GPT with 2-oxoglutaramate synthesis activity. Briefly, authentic 2-oxoglutarmate (structure confirmed with NMR) made by chemical synthesis to validate the HPLC assay, above, by confirming that the product of the assay (molecule synthesized in response to the expressed GPT) and the authentic 2-oxoglutaramate elute at the same retention time. In addition, when mixed together the assay product and the authentic compound elute as a single peak. Furthermore, the validation of the HPLC assay also included monitoring the disappearance of the substrate glutamine and showing that there was a 1:1 molar stoechiometry between glutamine consumed to 2-oxoglutaramte produced. The assay procedure always included two controls, one without the enzyme added and one without the glutamine added. The first shows that the production of the 2-oxoglutaramate was dependent upon having the enzyme present, and the second shows that the production of the 2-oxoglutaramate was dependent upon the substrate glutamine.

Results:

[0246] Expression of the rice GPT coding sequence of SEQ ID NO: 10 resulted in the over-expression of recombinant GPT protein having 2-oxoglutaramate synthesis-catalyzing bioactivity. Specifically, 1.72 nanomoles of 2-oxoglutaramate activity was observed in the *E. coli* cells overexpressing the recombinant rice GPT, compared to only 0.02 nanomoles of 2-oxoglutaramate activity in control *E. coli* cells, an 86-fold activity level increase over control.

Example 21

Isolation and Expression of Recombinant Soybean GPT Gene Coding Sequence and Analysis of Biological Activity

[0247] In this example, the putative coding sequence for soybean GPT was isolated and expressed in *E. coli*. Biologically active recombinant soybean GPT was produced, and catalyzed the increased synthesis of 2-oxoglutaramate, as confirmed by HPLC.

Materials and Methods:

[0248] Soybean GPT Coding Sequence and Expression in *E. coli:*

[0249] The soybean (*Glycine max*) GPT coding sequence was determined and synthesized, inserted into a PET28 vector, and expressed in *E. coli*. Briefly, *E. coli* cells were transformed with the expression vector and transformants grown overnight in LB broth diluted and grown to OD 0.4, expression induced with isopropyl-B-D-thiogalactoside (0.4 micro-

molar), grown for 3 hr and harvested. A total of 25×106 cells were then assayed for biological activity using the HPLC assay, below. Untransformed, wild type *E. coli* cells were assayed as a control. An additional control used *E. coli* cells transformed with an empty vector.

[0250] The DNA sequence of the soybean GPT coding sequence used in this example is provided in SEQ ID NO: 12, and the encoded GPT protein amino acid sequence is presented in SEQ ID NO: 13.

HPLC Assay for 2-Oxoglutaramate:

[0251] HPLC was used to determine 2-oxoglutaramate production in GPT-overexpressing *E. coli* cells, as described in Example 20, supra.

Results:

[0252] Expression of the soybean GPT coding sequence of SEQ ID NO: 12 resulted in the over-expression of recombinant GPT protein having 2-oxoglutaramate synthesis-catalyzing bioactivity. Specifically, 31.9 nanomoles of 2-oxoglutaramate activity was observed in the *E. coli* cells overexpressing the recombinant soybean GPT, compared to only 0.02 nanomoles of 2-oxoglutaramate activity in control *E. coli* cells, a nearly 1.600-fold activity level increase over control.

Example 22

Isolation and Expression of Recombinant Zebra Fish GPT Gene Coding Sequence and Analysis of Biological Activity

[0253] In this example, the putative coding sequence for Zebra fish GPT was isolated and expressed in *E. coli*. Biologically active recombinant Zebra fish GPT was produced, and catalyzed the increased synthesis of 2-oxoglutaramate, as confirmed by HPLC.

Materials and Methods:

[0254] Zebra Fish GPT Coding Sequence and Expression in *E. coli:*

[0255] The Zebra fish (*Danio rerio*) GPT coding sequence was determined and synthesized, inserted into a PET28 vector, and expressed in *E. coli*. Briefly, *E. coli* cells were transformed with the expression vector and transformants grown overnight in LB broth diluted and grown to OD 0.4, expression induced with isopropyl-B-D-thiogalactoside (0.4 micromolar), grown for 3 hr and harvested. A total of 25×106 cells were then assayed for biological activity using the HPLC assay, below. Untransformed, wild type *E. coli* cells were assayed as a control. An additional control used *E. coli* cells transformed with an empty vector.

[0256] The DNA sequence of the Zebra fish GPT coding sequence used in this example is provided in SEQ ID NO: 16, and the encoded GPT protein amino acid sequence is presented in SEQ ID NO: 17.

HPLC Assay for 2-Oxoglutaramate:

[0257] HPLC was used to determine 2-oxoglutaramate production in GPT-overexpressing *E. coli* cells, as described in Example 20, supra.

Results:

[0258] Expression of the Zebra fish GPT coding sequence of SEQ ID NO: 16 resulted in the over-expression of recombinant GPT protein having 2-oxoglutaramate synthesis-catalyzing bioactivity. Specifically, 28.6 nanomoles of 2-oxoglutaramate activity was observed in the *E. coli* cells overexpressing the recombinant Zebra fish GPT, compared to only 0.02 nanomoles of 2-oxoglutaramate activity in control *E. coli* cells, a more than 1.400-fold activity level increase over control.

Example 23

Generation and Expression of Recombinant Truncated *Arabidopsis* GPT Gene Coding Sequences and Analysis of Biological Activity

[0259] In this example, two different truncations of the *Arabidopsis* GPT coding sequence were designed and expressed in *E. coli*, in order to evaluate the activity of GPT proteins in which the putative chloroplast signal peptide is absent or truncated. Recombinant truncated GPT proteins corresponding to the full length *Arabidopsis* GPT amino acid sequence of SEQ ID NO: 2, truncated to delete either the first 30 amino-terminal amino acid residues, or the first 45 amino-terminal amino acid residues, were successfully expressed and showed biological activity in catalyzing the increased synthesis of 2-oxoglutaramate, as confirmed by HPLC.

Materials and Methods:

[0260] Truncated *Arabidopsis* GPT Coding Sequences and Expression in *E. coli:*

[0261] The DNA coding sequence of a truncation of the *Arabidopsis thaliana* GPT coding sequence of SEQ ID NO: 1 was designed, synthesized, inserted into a PET28 vector, and expressed in *E. coli*. The DNA sequence of the truncated *Arabidopsis* GPT coding sequence used in this example is provided in SEQ ID NO: 20 (−45 AA construct), and the corresponding truncated GPT protein amino acid sequence is provided in SEQ ID NO: 21. Briefly, *E. coli* cells were transformed with the expression vector and transformants grown overnight in LB broth diluted and grown to OD 0.4, expression induced with isopropyl-B-D-thiogalactoside (0.4 micromolar), grown for 3 hr and harvested. A total of 25×10^6 cells were then assayed for biological activity using HPLC as described in Example 20. Untransformed, wild type *E. coli* cells were assayed as a control. An additional control used *E. coli* cells transformed with an empty vector.

[0262] Expression of the truncated-45 *Arabidopsis* GPT coding sequence of SEQ ID NO: 20 resulted in the over-expression of biologically active recombinant GPT protein (2-oxoglutaramate synthesis-catalyzing bioactivity). Specifically, 16.1 nanomoles of 2-oxoglutaramate activity was observed in the *E. coli* cells overexpressing the truncated-45 GPT, compared to only 0.02 nanomoles of 2-oxoglutaramate activity in control *E. coli* cells, a more than 800-fold activity level increase over control. For comparison, the full length *Arabidopsis* gene coding sequence expressed in the same *E. coli* assay generated 2.8 nanomoles of 2-oxoglutaramate activity, or roughly less than one-fifth the activity observed from the truncated recombinant GPT protein.

Example 24

GPT+GS Transgenic Tobacco Seed Germination Tolerates High Salt Concentrations

[0263] In this example, seeds form the double transgene tobacco line XX-3 (Cross 3 in Table 4, see Example 7) were

tested in a seed germination assay designed to evaluate tolerance to high salt concentrations.

Materials and Methods:

[0264] Tobacco seeds from the wild type and XX-3 populations were surfaced sterilized (5% bleach solution for 5 minutes followed by a 10% ethanol wash for 3 minutes) and rinsed with sterile distilled water. The surface sterilized seeds were then spread on Murashige and Skoog media (10% agarose) without sucrose and containing either 0 or 200 mM NaCl. The seeds were allowed to germinate in darkness for 2 days followed by 6 days under a 16:8 photoperiod at 24° C. On day eight the rate of germination was determined by measuring the percentage of seeds from the control or transgene plants that had germinated.

Results:

[0265] The results are tabulated in Table XXI below. The rate of germination of the transgenic plant line seeds under zero salt conditions was the same as observed with wild type control plant seeds. In stark contrast, the germination rate of the transgenic plant line seeds under very high salt conditions far exceeded the rate seen in wild type control seeds. Whereas over 81% of the transgenic plant seeds had germinated under the high salt conditions, only about 9% of the wild type control plant seeds had germinated by the same time point. These data indicate that the transgenic seeds are capable of germinating very well under high salt concentrations, an important trait for plant growth in areas of increasingly high water and/or soil salinity.

TABLE XXI

TRANSGENIC TOBACCO PLANTS GERMINATE AND TOLERATE HIGH SALT

Plant type	Control (0 mM NaCl) % Germination	Test (200 mM NaCl)a % Germination
Wild type	92, 87, 94	9, 11, 8
Transgene line XX-3	92, 91, 94	84, 82, 78

Example 25

Method for Generating Transgenic Maize Plants Carrying *Hordeum* GPT and GS1 Transgenes

[0266] This example provides a method for generating transgenic maize plants expressing GPT and GS1 transgenes. Maize (*Zea mays*, hybrid line Hi-II) type II callus is biolistically transformed with an expression cassette comprising the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo small subunit promoter of SEQ ID NO: 39 (expression casette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SEQ ID NO: 44. Transformation of maize callus is achieved by particle bombardment.

Vector Constructs:

[0267] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn ubiquitin promoters, respectively, is cloned into the plasmid pAHC25 (Christensen and Quail, 1996, Transgenic Research 5:213-218) modified to include a bar gene conferring resistance to bialophos, or a similar vector, in order to generate the transgene expression vector.

Transformation and Regeneration:

[0268] The transgene expression vector is introduced into immature zygotic embryo source callus of parent maize hybrid line Hi-II (A188xB73 origin) (Armstrong et al., 1991, Maize Genetics Coop Newsletter 65:92-93) using particle bombardment, essentially as described (Frame et al., 2000, In Vitro Cell. Dev. Biol-Plant 36:21-29; this method was developed by and is routinely used at the Iowa State University Center for Plant Transformation).

[0269] More specifically, immature zygotic embryo source callus is prepared for transformation by serial culturing on a callus-initiating medium (N6E, Songstad et al., 1996, In vitro Cell Dev. Biol.-Plant 32:179-183). Washed gold particles are coated with the plasmid construct and used to bombard the callus with a PDS 1000/He biolistic gun as described (Sanford et al., 1993, Methods in Enzymology 217: 483-509). After 7-10 days on initiation medium, the callus is then transferred to selection medium containing bialophos (N6S, Songstad et al., 1996, supra) and allowed to grow. Following the development of bialophos resistant clones, callus pieces are transferred to a regeneration medium (Armstrong and Green, 1985, Planta 164:207-214) containing bialophos and allowed to grow for several weeks. Thereafter, the resulting plantlets are transferred to regeneration medium without the selection agent, and cultivated.

[0270] Transgenic corn plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 26

Method for Generating Transgenic Rice Plants Carrying *Hordeum* GPT and GS1 Transgenes

[0271] This example provides a method for generating transgenic rice plants expressing GPT and GS1 transgenes. Rice (*Oryza sativa*, Japonica cultivar Nipponbare) type II calus is transformed with the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo small subunit promoter of SEQ ID NO: 39 (expression cassette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SEQ ID NO: 44. Transformation is achieved by *Agrobacterium*-mediated transformation.

Vector Constructs:

[0272] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn ubiquitin promoters, respectively, is cloned into base vector pTF101.1, using standard molecular cloning methodologies, to generate the transgene expression vector. Base vector pTF101.1 is a derivative of the pPZP binary vector (Hajdukiewicz et al 1994, Plant Mol. Biol. 25:989-994), which includes the right and left T-DNA border

fragments from a nopaline strain of *A. tumefaciens*, a broad host origin of replication (pVS1) and a spectinomycin-resistant marker gene (aadA) for bacterial selection. The plant selectable marker gene cassette includes the phosphinothricin acetyl transferase (bar) gene from *Streptomyces hygroscopicus* that confers resistance to the herbicides glufosinate and bialophos. The soybean vegetative storage protein terminator (Mason et al., 1993) follows the 3' end of the bar gene.

Media:

[0273] YEP Medium: 5 g/L yeast extract, 10 g/L peptone, 5 g/L $NaCl_2$, 15 g/L Bacto-agar. pH to 6.8 with NaOH. After autoclaving, the appropriate antibiotics are added to the medium when it has cooled to 50° C.

[0274] Infection Medium: N6 salts and vitamins (Chu et al., 1975, Sci. Sinica 18: 659-668), 1.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 0.7 g/L L-proline, 68.4 g/L sucrose, and 36 g/L glucose (pH 5.2). This medium is filter-sterilized and stored at 4° C. Acetosyringone (AS, 100 μM) is added just prior to use (prepared from 100 μM stocks of filter-sterilized AS, dissolved in DMSO to 200 mM then diluted 1:1 with water).

[0275] Callus Induction Medium: N6 salts and vitamins, 300 mg/L casamino acids, 2.8 g/L L-proline, 30 g/L sucrose, and 4 g/L gelrite (pH 5.8). Filter sterilized N6 Vitamins and 2 mg/L 2,4-D, are added to this medium after autoclaving.

[0276] Co-cultivation Medium (make fresh): N6 salts and vitamins, 300 mg/L casamino acids, 30 g/L sucrose, 10 g/L glucose, and 4 g/L gelrite (pH 5.8). Filter sterilized N6 vitamins, acetosyringone (AS) 100 μM and 2 mg/L 2,4-D are added to this medium after autoclaving.

[0277] Selection Medium: N6 salts and vitamins, 300 mg/L casamino acids, 2.8 g/L L-proline, 30 g/L sucrose, and 4 g/L gelrite (pH 5.8). Filter sterilized N6 vitamins, 2 mg/L 2,4-D, 2 mg/L Bialaphos (Shinyo Sangyo, Japan) and 500 mg/L carbenicillin are added to this medium after autoclaving.

[0278] Regeneration Medium I: MS salts and vitamins (Murashige and Skoog, 1962), 2 g/L casamino acids, 30 g/L sucrose, 30 g/L sorbitol, and 4 g/L gelrite (pH 5.8). Filter sterilized MS vitamins, 100 mg/L cefotaxime, 100 mg/L vancomycin, 0.02 mg/L NAA (naphthaleneacetic acid), 2 mg/L kinetin (Toki, 1997, supra) and 2 mg/L Bialaphos are added to this medium after autoclaving.

[0279] Regeneration Medium II: MS Salts and vitamins, 100 mg/L myo-inositol, 30 g/L sucrose, 3 g/L gelrite, (pH 5.8).

Transformation and Regeneration:

[0280] Japonica rice cultivar Nipponbare is transformed with *Agrobacterium tumefaciens* strain EHA101 (Hood et al., 1986, J. Bacteriol. 168:1291-1301), transformed with the pTF101.1 transgene expression vector carrying the *hordeum* GS1+GPT expression cassette. The vector system pTF101.1 in EHA101 is maintained on YEP medium (An et al., 1988) containing 100 mg/L spectinomycin (for pTF101.1) and 50 mg/L kanamycin (for EHA101).

[0281] Briefly, callus tissue derived from the mature rice embryo is used as the starting material for transformation. Callus induction, co-cultivation, selection and regeneration I media are based on those of Hiei et al., 1994, The Plant Journal 6 (2):271-282.

[0282] More specifically, calli are induced as follows. First, 15-20 rice seeds are dehusked and rinsed in 10 ml of 70% Ethanol (50 ml conical tube) by vigorously shaking the tube for one minute, followed by rinsing once with sterile water. Then, 10 ml of 50% commercial bleach (5.25% hypochlorite) is added and placed on a shaker for 30 minutes (low setting). The bleach solution is then poured-off and the seeds rinsed five times with ~10 ml of sterilized water each time. With a small portion of the final rinse, the seeds are poured onto sterilized filter paper (in a sterile petri plate) and then allowed to dry. Using sterile forceps, several (i.e., 5) seeds are transferred to the surface of individual sterile petri plates containing callus induction medium. The plates are wrapped with vent tape and incubated in the light (16:8 photoperiod) at 29° C. Seeds are observed every few days and those showing signs of contamination are discarded.

[0283] After two to three weeks, developing callus is visible on the scutellum of the mature seed. Calli are then subcultured to fresh induction medium and allowed to proliferate. Four days prior to infection, the callus tissue is cut into 2-4 mm pieces and transferred to fresh induction medium.

[0284] The selection medium uses modifications from Toki (Toki, 1997, Plant Molecular Biology Reporter 15:16-21) whereby bialophos (2 mg/L) is employed for plant selection and carbenicillin (500 mg/L) for counter selection against *Agrobacterium*. Regeneration II medium is as described (Armstrong and Green, 1985, Planta 164:207-214).

[0285] *Agrobacterium* culture is grown (i.e., for 3 days at 19° C., or 2 days at 28° C.) on YEP medium amended with spectinomycin (100 mg/L) and kanamycin (50 mg/L). An aliquot of the culture is then suspended in ~15 ml of liquid infection medium supplemented with 100 μM AS in a 50 ml conical tube (no pre-induction). The optical density is adjusted to <0.1 (OD_{550}=0.06-0.08) before use.

[0286] For infection, rice calli are first placed into bacteria-free infection medium+AS (50 ml conical). This pre-wash is removed and replaced with 10 ml of the prepared *Agrobacterium* suspension (OD_{550}<0.1). Then, the conical is fastened onto a vortex shaker (low setting) for two minutes. After infection, calli are poured out of the conical onto a stack of sterile filter paper in a 100×15 petri dish to blot dry. Then, they are transferred off the filter paper and onto the surface of co-cultivation medium with sterile forceps. Co-cultivation plates are wrapped with vent tape and incubated in the dark at 25° C. for three days. After three days of co-cultivation, the calli are washed five times with 5 ml of the liquid infection medium (no AS) supplemented with carbenicillin (500 mg/L) and vancomycin (100 mg/L). Calli are blotted dry on sterile filter paper as before. Individual callus pieces are transferred off the paper and onto selection medium containing 2 mg/L bialaphos. Selection plates are wrapped with parafilm and placed in the light at 29° C.

[0287] For selection of stable transformation events, plant tissue is cultured onto fresh selection medium every two weeks. This should be done with the aid of a microscope to look for any evidence of *Agrobacterium* overgrowth. If overgrowth is noted, the affected calli should be avoided (contaminated calli should not be transferred). The remaining tissue is then carefully transferred, preferably using newly sterilized forceps for each calli. Putative clones begin to appear after six to eight weeks on selection. A clone is recognized as white, actively growing callus and is distinguishable from the brown, unhealthy non-transformed tissue. Individual transgenic events are identified and the white, actively growing tissue is transferred to individual plates in order to produce enough tissue to take to regeneration. Regeneration

of transgenic plants is accomplished by selecting new lobes of growth from the callus tissue and transferring them onto Regeneration Medium I (light, 25° C.). After two to three weeks, the maturing tissue is transferred to Regeneration Medium II for germination (light, 25° C.). When the leaves are approximately 4-6 cm long and have developed good-sized roots, the plantlets may be transferred (on an individual basis, typically 7-14 days after germination begins) to soilless mix using sterile conditions.

[0288] Transgenic rice plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 27

Method for Generating Transgenic Sugarcane Plants Carrying *Hordeum* GPT and GS1 Transgenes

[0289] This example provides a method for generating transgenic sugarcane plants expressing GPT and GS1 transgenes. Sugarcane (*Saccharum* spp L) is biolistically transformed with an expression cassette comprising the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo small subunit promoter of SEQ ID NO: 39 (expression cassette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SEQ ID NO: 44. Transformation of sugarcane callus is achieved by particle bombardment.

Vector Constructs:

[0290] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn ubiquitin promoters, respectively, are cloned into a small plasmid well established for sugarcane expression, such as pAHC20 (Thomson et al., 1987, EMBO J. 6:2519-2523), using standard molecular cloning methodologies, to generate the transgene expression vector. The plasmid used contains a selectable marker against either the phospinothricin family of herbicides or the antibiotics geneticin or kanamycin, each of which have been shown effective (Ingelbrecht et al., 1999, Plant Physiology 119:1187-1197; Gallo-Maegher & Irvine, 1996, Crop Science 36:1367-1374).

Transformation and Regeneration:

[0291] The plasmid containing the expression cassette encoding the *hordeum* GS1 and GPT coding sequences is introduced into embryogenic callus prepared for transformation by the basic method of Gallo-Maegher and Irvine (Gallo-Maegher and Irvine, 1996, supra) and Ingelbrecht et al. (Ingelbrecht et al., 1999, supra) with the improved stimulation of shoot regeneration with thidiazuron (Gallo-Maegher et al., 2000, In vitro Cell Dev. Biol.-Plant 36:37-40). This particle bombardment method is effective in transforming sugarcane (see, for example, Gilbert et al., 2005, Crop Science 45:2060-2067; and see the foregoing references). Regenerable sugarcane varieties, such as the commercial varieties CP65-357 and CP72-1210, may be used to generate transgene events.

[0292] Briefly, 7- to 40-week old calli are bombarded with plasmid-coated tungsten or gold particles. Two days after bombardment the calli are transferred to selection medium. Four weeks later the resistant calli are transferred to shoot-induction medium containing the selection agent and sub-cultured every two weeks for approximately 12 weeks, at which time the shoots are transferred to Magenta boxes containing rooting medium with selection agent. The shoots are maintained on this medium for approximately 8 weeks, at which time those with good root development are transferred to potting mix and the adapted to atmospheric growth.

[0293] Transgenic sugarcane plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 28

Method for Generating Transgenic Wheat Plants Carrying *Hordeum* GPT And GS1 Transgenes

[0294] This example provides a method for generating transgenic wheat plants expressing

[0295] GPT and GS1 transgenes. Wheat (*Triticum* spp.) is biolistically transformed with an expression cassette comprising the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo small subunit promoter of SEQ ID NO: 39 (expression cassette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SEQ ID NO: 44. Transformation of wheat callus is achieved by particle bombardment.

Vector Constructs:

[0296] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn (maize) ubiquitin promoters, respectively, are cloned into a plasmid such as pAHC17, which contains the bar gene to provide the desired resistance to the phosphinothricin-class of herbicides for selection of transformants, using standard molecular cloning methodologies, to generate the transgene expression vector.

Transformation and Regeneration:

[0297] Wheat is transformed biolistically, and transgenic events regenerated, essentially as described (Weeks et al., 1993, Plant Physiology. 102:1077-1084; Blechl and Anderson, 1996, Nat. Biotech. 14:875-879; Okubara et. al., 2002, Theoretical and Applied Genetics. 106:74-83). These methods were developed and are routinely practiced at the US Department of Agriculture, Agricultural Research Service, Western Regional Research Center (Albany Calif.). The highly regenerable hexaploid spring wheat cultivar 'Bobwhite' is used as the source of immature embryos for bombardment with plasmid-coated particles.

[0298] Bombarded embryos are cultured without selection for 1-3 weeks in the dark on MS media before transferring them to shoot induction medium (MS media plus hormones and selection agent bialophos (1, 1.5, 2, 3 mg/L) for 2-8 weeks with subculturing weekly (Blechl et al., 2007, J Cereal

Science 45:172-183). Shoots that formed are transferred to rooting medium also containing the selection agent (bialophos 3 mg/L) (Weeks et al., 1993, supra). Well-rooted plantlets are transferred to potting media and adapted to atmospheric growth conditions.

[0299] Transgenic wheat plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 29

Method for Generating Transgenic Sorghum Plants Carrying *Hordeum* GPT and GS1 Transgenes

[0300] This example provides a method for generating transgenic sorghum plants expressing GPT and GS1 transgenes. *Sorghum* (*Sorghum* spp L) is transformed with *Agrobacterium* carrying an expression cassette encoding the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo subunit promoter of SEQ ID NO: 39 (expression cassette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SE ID NO: 44.

Vector Constructs:

[0301] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn ubiquitin promoters, respectively, is cloned into a stable binary vector such as pZY101 (Vega et al 2008, Plant Cell Rep. 27:297-305), using standard molecular cloning methodologies, to generate the transgene expression vector.

Transformation and Regeneration:

[0302] *Agrobacterium*-mediated transformation and recovery Of transgenic sorghum plants is as described (Lu et al., 2009, Plant Cell Tissue Organ Culture 99:97-108). These methods are routinely used by the University of Missouri Plant Transformation Core Facility. The public sorghum line, P898012, is grown as described (Lu et al., 2009, supra) and transformed with *Agrobacterium tumefaciens* strain EHA101 (Hood et al., 1986, supra) transformed with the transgene expression vector.

[0303] More specifically, Agrobacterium (0.3-0.4 OD) harboring the transgene expression vector is used to inoculate immature sorghum embryos for 5 minutes. The embryos are then transferred onto filter paper on top of their co-cultivation medium, containing acetosyringone to enhance the effectiveness of the infection. Embryos are incubated for 3-5 days and then transferred for another 4 days on resting medium (containing carbenicillin) and then transferred onto callus induction medium (with selection agent PPT) with weekly transfers. Once somatic embyrogenic cells develop they are transferred onto shooting medium (with carbenicillin and PPT) until shoots (2-5 cm long) develop. Shoots are transferred to Magenta boxes with rooting medium (with PPT) and maintained in 16 h light and 8 h darkness until 8-20 cm tall well-rooted plantlets are produced. They are then transferred to potting mix and adapted to atmospheric conditions.

[0304] Transgenic sorghum plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 30

Method for Generating Transgenic Switchgrass Plants Carrying *Hordeum* GPT and GS1 Transgenes

[0305] This example provides a method for generating transgenic switchgrass plants expressing GPT and GS1 transgenes. Switchgrass (*Panicum virgatum*) is transformed with *Agrobacterium* carrying a transgene expression vector including an expression cassette encoding the *hordeum* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 40 under the control of the rice RuBisCo small subunit promoter of SEQ ID NO: 39 (expression cassette of SEQ ID NO: 42), and the *hordeum* GPT coding sequence of SEQ ID NO: 45 under the control of the corn ubiquitin (Ubil) promoter of SE ID NO: 44.

Vector Constructs:

[0306] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the rice RuBisCo small subunit and corn (maize) ubiquitin promoters, respectively, is cloned into a Cambia vector thirteen hundred series (i.e., 1305.1) containing the HPT gene which provides hygromycin resistance for selection of the Switchgrass events, using standard molecular cloning methodologies, to generate the transgene expression vector.

Transformation and Regeneration:

[0307] *Agrobacterium*-mediated transformation and recovery of transgenic switchgrass plants is essentially as described (Somleva et al., 2002, Crop Science 42:2080-2087; Somleva 2006, Switchgrass (*Panicum virgatum* L.) In Methods in Molecular Biology Vol 344. *Agrobacterium* Protocols 2/e, Volume 2. Ed K. Wang Humana Press Inc., Totowa, N.J.; Xi et al 2009, Bioengineering Research 2:275-283). These methods are routinely used by the Plant Biotechnology Resource and Outreach Center at Michigan State University.

[0308] Briefly, explants of embryonic callus from the mature caryopses of the public Switchgrass cv. Alamo are transformed with *Agrobacterium tumefaciens* strain EHA105 (Hood et al., 1986, supra) carrying the transgene expression vector. *Agrobacterium* (0.8-1.0 OD) harboring the transgene expression vector and pretreated with acetosynringone is used to inoculate the switchgrass callus for 10 minutes and then co-cultivated for 4-6 days in the dark. The explants are then washed free of the *agrobacterium* and placed on selection medium containing the antibiotic timentin and hygromycin; selection requires 2-6 months. Subculturing is carried out at 4-week intervals. Regeneration is accomplished in 4-8 weeks on media containing GA3, timentin and hygromycin under a photoperiod of 16 h light and 8 dark. The plantlets are then transferred to Magenta boxes with regeneration medium containing GA3, timentin and hygromycin for another 4

weeks as before. The plants are then transferred to soil and adapted to atmospheric growth.

[0309] Transgenic switchgrass plants may be grown and evaluated through maturity, and seeds harvested for use in generating subsequent generations of an event. Various phenotypic characteristics may be observed in T_0 events, as well as in T_1 and subsequent generations, and used to select seed sources for the development of subsequent generations. High performing lines may be selfed to achieve trait homozygosity and/or crossed.

Example 31

Method for Generating Transgenic Soybean Plants Carrying *Arabidopsis* GPT and GS1 Transgenes

[0310] This example provides a method for generating transgenic soybean plants expressing GPT and GS1 transgenes. Soybean (*Glycine max*) is transformed with *Agrobacterium* carrying a transgene expression vector including an expression cassette encoding the *Arabidopsis* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 7 under the control of the tomato RuBisCo small subunit promoter of SEQ ID NO: 22 (expression cassette of SEQ ID NO: 47), and the *Arabidopsis* GPT coding sequence of SEQ ID NO: 1 under the control of the 35S cauliflower mosaic virus (CMV) promoter (expression cassette of SEQ ID NO: 27).

Vector Constructs:

[0311] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the tomato RuBisCo small subunit and 35S CMV promoters, respectively, is cloned into pTF101.1, using standard molecular cloning methodologies, to generate the transgene expression vector. pTF101.1 is a derivative of the pPZP binary vector (Hajdukiewicz et al 1994, Plant Mol. Biol. 25:989-994), which includes the right and left T-DNA border fragments from a nopaline strain of *A. tumefaciens*, a broad host origin of replication (pVS1) and a spectinomycin-resistant marker gene (aadA) for bacterial selection. The plant selectable marker gene cassette includes the phosphinothricin acetyl transferase (bar) gene from *Streptomyces hygroscopicus* that confers resistance to the herbicides glufosinate and bialophos. The soybean vegetative storage protein terminator (Mason et al., 1993) follows the 3' end of the bar gene.

Media:

[0312] YEP Solid Medium: 5 g/L Yeast extract, 10 g/L Peptone, 5 g/L $NaCl_2$, 12 g/L Bacto-agar. pH to 7.0 with NaOH. Appropriate antibiotics should be added to the medium after autoclaving. Pour into sterile 100×15 plates (~25 ml per plate).

[0313] YEP Liquid Medium: 5 g/L Yeast extract, 10 g/L Peptone, 5 g/L $NaCl_2$. pH to 7.0 with NaOH. Appropriate antibiotics should be added to the medium prior to inoculation.

[0314] Co-cultivation Medium: 1/10× B5 major salts, 1/10× B5 minor salts, 2.8 mg/L Ferrous, 3.8 mg/L NaEDTA, 30 g/L Sucrose, 3.9 g/L MES, and 4.25 g/L Noble agar (pH 5.4). Filter sterilized 1× B5 vitamins, GA3 (0.25 mg/L), BAP (1.67 mg/L), Cysteine (400 mg/L), Dithiothrietol (154.2 mg/L), and 40 mg/L acetosyringone are added to this medium after autoclaving. Pour into sterile 100×15 mm plates (~88 plates/L). When solidified, overlay the co-cultivation medium with sterile filter paper to reduce bacterial overgrowth during co-cultivation (Whatman #1, 70 mm).

[0315] Infection Medium: 1/10× B5 major salts, 1/10× B5 minor salts, 2.8 mg/L Ferrous, 3.8 mg/L NaEDTA, 30 g/L Sucrose, 3.9 g/L MES (pH 5.4). Filter sterilized 1× B5 vitamins, GA3 (0.25 mg/L), BAP (1.67 mg/L), and 40 mg/L acetosyringone are added to this medium after autoclaving.

[0316] Shoot Induction Washing Medium: 1× B5 major salts, 1× B5 minor salts, 28 mg/L Ferrous, 38 mg/L NaEDTA, 30 g/L Sucrose, and 0.59 g/L MES (pH 5.7). Filter sterilized 1× B5 vitamins, BAP (1.11 mg/L), Timentin (100 mg/L), Cefotaxime (200 mg/L), and Vancomycin (50 mg/L) are added to this medium after autoclaving.

[0317] Shoot Induction Medium I: 1× B5 major salts, 1× B5 minor salts, 28 mg/L Ferrous, 38 mg/L NaEDTA, 30 g/L Sucrose, 0.59 g/L MES, and 7 g/L Noble agar (pH 5.7). Filter sterilized 1× B5 vitamins, BAP (1.11 mg/L), Timentin (50 mg/L), Cefotaxime (200 mg/L), and Vancomycin (50 mg/L) are added to this medium after autoclaving. Pour into sterile 100×20 mm plates (26 plates/L).

[0318] Shoot Induction Medium II: 1× B5 major salts, 1× B5 minor salts, 28 mg/L Ferrous, 38 mg/L NaEDTA, 30 g/L Sucrose, 0.59 g/L MES, and 7 g/L Noble agar (pH 5.7). Filter sterilized 1× B5 vitamins, BAP (1.11 mg/L), Timentin (50 mg/L), Cefotaxime (200 mg/L), Vancomycin (50 mg/L) and Glufosinate (6 mg/L) are added to this medium after autoclaving. Pour into sterile 100×20 mm plates (26 plates/L).

[0319] Shoot Elongation Medium: 1×MS major salts, 1×MS minor salts, 28 mg/L Ferrous, 38 mg/L NaEDTA, 30 g/L Sucrose, 0.59 g/L MES, and 7 g/L Noble agar (pH 5.7). Filter sterilized 1× B5 vitamins, Asparagine (50 mg/L), L-Pyroglutamic Acid (100 mg/L), IAA (0.1 mg/L), GA3 (0.5 mg/L), Zeatin-R (1 mg/L), Timentin (50 mg/L), Cefotaxime (200 mg/L), Vancomycin (50 mg/L), and Glufosinate (6 mg/L) are added to this medium after autoclaving. Pour into sterile 100×25 mm plates (22 plates/L).

[0320] Rooting Medium: 1× MS major salts, 1×MS minor salts, 28 mg/L Ferrous, 38 mg/L NaEDTA, 20 g/L Sucrose, 0.59 g/L MES, and 7 g/L Noble agar (pH 5.6). Filter sterilized 1× B5 vitamins, Asparagine (50 mg/L), and L-Pyroglutamic Acid (100 mg/L) are added to this medium after autoclaving. Pour into sterile 150×25 mm vial (10 ml/vial).

Transformation and Regeneration:

[0321] *Agrobacterium* cultures are prepared for infecting seed explants as follows. The vector system, pTF102 in EHA101, is cultured on YEP medium (An et al., 1988) containing 100 mg/L spectinomycin (for pTF102), 50 mg/L kanamycin (for EHA101), and 25 mg/L chloramphenicol (for EHA101). 24 hours prior to infection a 2 ml culture of *Agrobacterium* is started by inoculating a loop of bacteria from the fresh YEP plate in YEP liquid medium amended with antibiotics. This culture is allowed to grow to saturation (8-10 hours) at 28° C. in a shaker incubator (–250 rpm). Then 0.2 ml of starter culture is transferred to a 1 L flask containing 250 ml of YEP medium amended with antibiotics. The culture is allowed to grow overnight at 28° C., 250 rpm to log phase (OD650=0.3-0.6 for EHA105) or late log phase ($OD_{650=1.0}$-1.2 for EHA101). The *Agrobacterium* culture is then pelleted at 3,500 rpm for 10 minutes at 20° C., and the pellet resuspended in infection medium by pipetting through the pellet. Bacterial cell densities are adjusted to a final OD650=0.6 (for

EHA105) or OD650=0.6 to 1.0 (for EHA101). *Agrobacteria*-containing infection medium is shaken at 60 rpm for at least 30 minutes before use.

[0322] Explants are prepared for inoculation as follows. Seeds are sterilized, ideally with a combination of bleach solution and exposure to chlorine gas. Prior to infection, (−20 hours), sees are imbibed with deionized sterile water in the dark. Imbibed soybean seeds are transferred to a sterile 100× 15 petri plate for dissection. Using a scalpel (i.e., #15 blade), longitudinal cuts are made along the hilum to separate the cotyledons and remove the seed coat. The embryonic axis found at the nodal end of the cotyledons is excised, and any remaining axial shoots/buds attached to the cotyledonary node are also removed.

[0323] *Agrobacterium*-mediated transformation is conducted as follows. Half-seed explants are dissected into a 100×25 mm petri plate and 30 ml *Agrobacterium*-containing infection media added thereto, such that the explants are completely covered by the infection media. Explants are allowed to incubate at room temperature for a short period of time (i.e., 30 minutes), preferably with occasional gentle agitation.

[0324] After infection, the explants are transferred to co-cultivation medium, preferably so that the flat, axial side is touching the filter paper. These plates are typically wrapped in parafilm, and cultivated for 5 days at 24° C. under an 18:6 photoperiod. Following this co-cultivation, shoot growth is induced by first washing the explants in shoot induction washing medium at room temperature, followed by placing the explants in shoot induction medium I, such that the explants are oriented with the nodal end of the cotyledon imbedded in the medium and the regeneration region flush to the surface with flat side up (preferably at a 30-45° angle). Explants are incubated at 24° C., 18:6 photoperiod, for 14 days. Explants are thereafter transferred to shoot induction medium II and maintained under the same conditions for another 14 days.

[0325] Following shoot induction, explants are transferred to shoot elongation medium, as follows. First, cotyledons are removed from the explants. A fresh cut at the base of the shoot pad flush to the medium is made, and the explants transferred to shoot elongation medium (containing glufosinate) and incubated at 24° C., 18:6 photoperiod, for 2-8 weeks. Preferably, explant tissue is transferred to fresh shoot elongation medium every 2 weeks, and at transfer, a fresh horizontal slice at the base of the shoot pad is made.

[0326] When shoots surviving the glufosinate selection have reached ~3 cm length, they are excised from the shoot pad, briefly dipped in indole-3-butyric acid (1 mg/ml, 1-2 minutes), then transferred to rooting medium for acclimatization (i.e., in 150×25 mm glass vials with the stems of the shoots embedded approximately ½ cm into the media). When well rooted, the shoots are transferred to soil and plantlets grown at 24° C., 18:6 photoperiod, for at least one week, watering as needed. When the plantlets have at least two healthy trifoliates, an herbicide paint assay may be applied to confirm resistance to glufosinate. Briefly, using a cotton swab, Liberty herbicide (150 mg l-1) is applied to the upper leaf surface along the midrib of two leaves on two different trifoliates. Painted plants are transferred to the greenhouse and covered with a humidome. Plantlets are scored 3-5 days after painting. Resistant plantlets may be transplanted immediately to larger pots (i.e., 2 gal).

Example 32

Method for Generating Transgenic Potato Plants Carrying *Arabidopsis* GPT and GS1 Transgenes

[0327] This example provides a method for generating transgenic potato plants expressing GPT and GS1 transgenes. Potato (*Solanum tuberosum*, cultivar Desiree) is transformed with *Agrobacterium* carrying a transgene expression vector including an expression cassette encoding the *Arabidopsis* glutamine synthetase (GS1) coding sequence of SEQ ID NO: 7 under the control of the tomato RuBisCo small subunit promoter of SEQ ID NO: 22 (expression cassette of SEQ ID NO: 47), and the *Arabidopsis* GPT coding sequence of SEQ ID NO: 1 under the control of the 35S cauliflower mosaic virus (CMV) promoter (expression cassette of SEQ ID NO: 27).

Vector Constructs:

[0328] An expression cassette comprising the *hordeum* GS1 and GPT genes, under the control of the tomato RuBisCo small subunit and 35S CMV promoters, respectively, is cloned into the Cambia 2201 vector which provides kanamycin resistance.

Transformation and Regeneration:

[0329] A suitable *Agrobacterium tumefaciens* strain such as UC-Riverside Agro-1 strain is employed and used for infecting potato explant tissue (see, Narvaez-Vasquez et al., 1992, Plant Mo. Biol. 20:1149-1157). Cultures are maintained at 28° C. in liquid medium containing 10 g/L Yeast extract, 10 g/L Peptone, 5 g/L $NaCl_2$, 10 mg/L kanamycin, 30 mg/L tetracycline, and 9.81 g/L Acetosyringone (50 mM). Overnight cultures are diluted with liquid MS medium (4.3 g/L MS salts, 20 g/L sucrose, 1 mg/L thiamine, 100 mg/L inositol and 7 g/L phytoagar, pH to 5.8.) to 10^8 *Agrobacterium* cells/ml for the infection of plant tissues (co-cultivation).

[0330] Potato leaf discs or tuber discs may be used as the explants to be inoculated. Discs are pre-conditioned by incubation on feeder plates for two to three days at 25° C. under dark conditions. Pre-conditioned explants are infected with *Agrobacterium* by soaking in 20 ml of sterile liquid MS medium (supra), containing 10^8 *Agrobacterium* cells/ml for about 20 minutes. Before or during the co-cultivation, the explants are carefully punched with a syringe needle, or scalpel blade. Then, the explants are blotted dry with sterile filter paper, and incubated again in feeder plates for another two days. Explants are then transferred to liquid medium with transgene-transformed *Agrobacterium*, and incubated for three days at 28° C. under dark conditions for calli and shoot development (development (2-4 cm) in the presence of kanamycin (100 mg/L).

[0331] Following co-cultivation, supra, the explants are washed three times with sterile liquid medium and finally rinsed with the same medium containing 500 mg/l of cefotaxime. The explants are blotted dry with sterile filter paper and placed on shoot induction medium (4.3 g/L MS salts, 10 mg/L thiamine, 1 mg/L nicotinic acid, 1 mg/L pyridxine, 100 mg/L inositol, 30 g/L sucrose, 1 mg/L zeatin, 0.5 mg/L IAA, 7 g/L phytoagar, 250 mg/L Cefotaxime, 500 mg/L Carbeni-

cillin, 100 mg/L Kanamycin) for 4-6 weeks. Thereafter, plantlets are transferred to rooting medium (4.3 g/L MS salts, 10 mg/L thiamine, 1 mg/L nicotinic acid, 1 mg/L pyridxine, 100 mg/L inositol, 20 g/L sucrose, 50 μg/L IAA, 7 g/L phytoagar, 50 mg/L Kanamycin and 500 mg/L Vancomycin) for 3-4 weeks.

[0332] All publications, patents, and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0333] The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any which are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

SEQ ID NO: 1 *Arabidopsis* glutamine phenylpyruvate transaminase DNA coding sequence:

ATGTACCTGGACATAAATGGTGTGATGATCAAACAGTTTAGCTTCAAAGCCTCTC

TTCTCCCATTCTCTTCTAATTTCCGACAAAGCTCCGCCAAAATCCATCGTCCTAT

CGGAGCCACCATGACCACAGTTTCGACTCAGAACGAGTCTACTCAAAAACCCGT

CCAGGTGGCGAAGAGATTAGAGAAGTTCAAGACTACTATTTTCACTCAAATGAG

CATATTGGCAGTTAAACATGGAGCGATCAATTTAGGCCAAGGCTTTCCCAATTTC

GACGGTCCTGATTTTGTTAAAGAAGCTGCGATCCAAGCTATTAAAGATGGTAAAA

ACCAGTATGCTCGTGGATACGGCATTCCTCAGCTCAACTCTGCTATAGCTGCGC

GGTTTCGTGAAGATACGGGTCTTGTTGTTGATCCTGAGAAAGAAGTTACTGTTAC

ATCTGGTTGCACAGAAGCCATAGCTGCAGCTATGTTGGGTTTAATAAACCCTGG

TGATGAAGTCATTCTCTTTGCACCGTTTTATGATTCCTATGAAGCAACACTCTCTA

TGGCTGGTGCTAAAGTAAAAGGAATCACTTTACGTCCACCGGACTTCTCCATCC

CTTTGGAAGAGCTTAAAGCTGCGGTAACTAACAAGACTCGAGCCATCCTTATGA

ACACTCCGCACAACCCGACCGGGAAGATGTTCACTAGGGAGGAGCTTGAAACC

ATTGCATCTCTCTGCATTGAAAACGATGTGCTTGTGTTCTCGGATGAAGTATACG

ATAAGCTTGCGTTTGAAATGGATCACATTTCTATAGCTTCTCTTCCCGGTATGTA

TGAAAGAACTGTGACCATGAATTCCCTGGGAAAGACTTTCTCTTTAACCGGATG

GAAGATCGGCTGGGCGATTGCGCCGCCTCATCTGACTTGGGGAGTTCGACAAG

CACACTCTTACCTCACATTCGCCACATCAACACCAGCACAATGGGCAGCCGTTG

CAGCTCTCAAGGCACCAGAGTCTTACTTCAAAGAGCTGAAAAGAGATTACAATG

TGAAAAAGGAGACTCTGGTTAAGGGTTTGAAGGAAGTCGGATTTACAGTGTTCC

CATCGAGCGGGACTTACTTTGTGGTTGCTGATCACACTCCATTTGGAATGGAGA

ACGATGTTGCTTTCTGTGAGTATCTTATTGAAGAAGTTGGGGTCGTTGCGATCC

CAACGAGCGTCTTTTATCTGAATCCAGAAGAAGGGAAGAATTTGGTTAGGTTTG

CGTTCTGTAAAGACGAAGAGACGTTGCGTGGTGCAATTGAGAGGATGAAGCAG

AAGCTTAAGAGAAAAGTCTGA

SEQ ID NO: 2 *Arabidopsis* GPT amino acid sequence

MYLDINGVMIKQFSFKASLLPFSSNFRQSSAKIHRPIGATMTTVSTQNESTQKPVQV

AKRLEKFKTTIFTQMSILAVKHGAINLGQGFPNFDGPDFVKEAAIQAIKDGKNQYARG

YGIPQLNSAIAARFREDTGLVVDPEKEVTVTSGCTEAIAAAMLGLINPGDEVILFAPFY

DSYEATLSMAGAKVKGITLRPPDFSIPLEELKAAVTNKTRAILMNTPHNPTGKMFTRE

ELETIASLCIENDVLVFSDEVYDKLAFEMDHISIASLPGMYERTVTMNSLGKTFSLTG

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WKIGWAIAPPHLTWGVRQAHSYLTFATSTPAQWAAVAALKAPESYFKELKRDYNVK

KETLVKGLKEVGFTVFPSSGTYFVVADHTPFGMENDVAFCEYLIEEVGVVAIPTSVF

YLNPEEGKNLVRFAFCKDEETLRGAIERMKQKLKRKV

SEQ ID NO: 3 Alfalfa GS1 DNA coding sequence (upper case) with 5' and 3' untranslated sequences (indicated in lower case).
atttccgttttcgttttcatttgattcattgaatcaaatcgaatcgaatctttaggattcaatacag

attccttagattttactaagtttgaaaccaaaaccaaaacATGTCTCTCCTTTCAGAT

CTTATCAACCTTGACCTCTCCGAAACCACCGAGAAAATCATCGCCG

AATACATATGGATTGGTGGATCTGGTTTGGACTTGAGGAGCAAAGC

AAGGACTCTACCAGGACCAGTTACTGACCCTTCACAGCTTCCCAAG

TGGAACTATGATGGTTCCAGCACAGGTCAAGCTCCTGGAGAAGAT

AGTGAAGTTATTATCTACCCACAAGCCATTTTCAAGGACCCATTTA

GAAGGGGTAACAATATCTTGGTTATGTGTGATGCATACACTCCAGC

TGGAGAGCCCATTCCCACCAACAAGAGACATGCAGCTGCCAAGAT

TTTCAGCCATCCTGATGTTGTTGCTGAAGTACCATGGTATGGTATT

GAGCAAGAATACACCTTGTTGCAGAAAGACATCAATTGGCCTCTTG

GTTGGCCAGTTGGTGGTTTTCCTGGACCTCAGGGACCATACTATTG

TGGAGCTGGTGCTGACAAGGCATTTGGCCGTGACATTGTTGACTC

ACATTACAAAGCCTGTCTTTATGCCGGCATCAACATCAGTGGAATC

AATGGTGAAGTGATGCCTGGTCAATGGGAATTCCAAGTTGGTCCCT

CAGTTGGTATCTCTGCTGGTGATGAGATATGGGTTGCTCGTTACAT

TTTGGAGAGGATCACTGAGGTTGCTGGTGTGGTGCTTTCCTTTGAC

CCAAAACCAATTAAGGGTGATTGGAATGGTGCTGGTGCTCACACAA

ATTACAGCACCAAGTCTATGAGAGAAGATGGTGGCTATGAAGTCAT

CTTGAAAGCAATTGAGAAGCTTGGGAAGAAGCACAAGGAGCACAT

TGCTGCTTATGGAGAAGGCAACGAGCGTAGATTGACAGGGCGACA

TGAGACAGCTGACATTAACACCTTCTTATGGGGTGTTGCAAACCGT

GGTGCGTCGATTAGAGTTGGAAGGGACACAGAGAAAGCAGGGAAA

GGTTATTTCGAGGATAGGAGGCCATCATCTAACATGGATCCATATG

TTGTTACTTCCATGATTGCAGACACCACCATTCTCTGGAAACCATA

Agccaccacacacacatgcattgaagtatttgaaagtcattgttgattccgcattagaatttgg

tcattgtttttctaggatttggatttgtgttattgttatggttcacactttgtttgtttgaatttgaggc

cttgttataggtttcatatttctttctcttgttctaagtaaatgtcagaataataatgtaat

SEQ ID NO: 4 Alfalfa GS1 amino acid sequence
MSLLSDLINLDLSETTEKIIAEYIWIGGSGLDLRSKARTLPGPVTDPSQLPKWNYDGS

STGQAPGEDSEVIIYPQAIFKDPFRRGNNILVMCDAYTPAGEPIPTNKRHAAAKIFSH

PDVVAEVPWYGIEQEYTLLQKDINWPLGWPVGGFPGPQGPYYCGAGADKAFGRDI

VDSHYKACLYAGINISGINGEVMPGQWEFQVGPSVGISAGDEIWVARYILERITEVA

GVVLSFDPKPIKGDWNGAGAHTNYSTKSMREDGGYEVILKAIEKLGKKHKEHIAAYG

-continued

EGNERRLTGRHETADINTFLWGVANRGASIRVGRDTEKAGKGYFEDRRPSSNMDP

YVVTSMIADTTILWKP

SEQ ID NO: 5 Alfalfa GS1 DNA coding sequence (upper case) with 5' and 3' untranslated sequences (indicated in lower case) and vector sequences from ClaI to SmaI/SspI and SspI/SmaI to SalI/XhoI (lower case, underlined).

atcgatgaattcgagctcggtacccatttccgttttcgttttcatttgattcattgaatcaaatcga

atcgaatctttaggattcaatacagattccttagattttactaagtttgaaaccaaaaccaaaa

cATGTCTCTCCTTTCAGATCTTATCAACCTTGACCTCTCCGAAACCA

CCGAGAAAATCATCGCCGAATACATATGGATTGGTGGATCTGGTTT

GGACTTGAGGAGCAAAGCAAGGACTCTACCAGGACCAGTTACTGA

CCCTTCACAGCTTCCCAAGTGGAACTATGATGGTTCCAGCACAGGT

CAAGCTCCTGGAGAAGATAGTGAAGTTATTATCTACCCACAAGCCA

TTTTCAAGGACCCATTTAGAAGGGGTAACAATATCTTGGTTATGTG

TGATGCATACACTCCAGCTGGAGAGCCCATTCCCACCAACAAGAG

ACATGCAGCTGCCAAGATTTTCAGCCATCCTGATGTTGTTGCTGAA

GTACCATGGTATGGTATTGAGCAAGAATACACCTTGTTGCAGAAAG

ACATCAATTGGCCTCTTGGTTGGCCAGTTGGTGGTTTTCCTGGACC

TCAGGGACCATACTATTGTGGAGCTGGTGCTGACAAGGCATTTGG

CCGTGACATTGTTGACTCACATTACAAAGCCTGTCTTTATGCCGGC

ATCAACATCAGTGGAATCAATGGTGAAGTGATGCCTGGTCAATGGG

AATTCCAAGTTGGTCCCTCAGTTGGTATCTCTGCTGGTGATGAGAT

ATGGGTTGCTCGTTACATTTTGGAGAGGATCACTGAGGTTGCTGGT

GTGGTGCTTTCCTTTGACCCAAAACCAATTAAGGGTGATTGGAATG

GTGCTGGTGCTCACACAAATTACAGCACCAAGTCTATGAGAGAAGA

TGGTGGCTATGAAGTCATCTTGAAAGCAATTGAGAAGCTTGGGAAG

AAGCACAAGGAGCACATTGCTGCTTATGGAGAAGGCAACGAGCGT

AGATTGACAGGGCGACATGAGACAGCTGACATTAACACCTTCTTAT

GGGGTGTTGCAAACCGTGGTGCGTCGATTAGAGTTGGAAGGGACA

CAGAGAAAGCAGGGAAAGGTTATTTCGAGGATAGGAGGCCATCAT

CTAACATGGATCCATATGTTGTTACTTCCATGATTGCAGACACCAC

CATTCTCTGGAAACCATAAgccaccacacacacatgcattgaagtatttgaaagtc

attgttgattccgcattagaatttggtcattgtttttctaggatttggatttgtgttattgttatggttc

acactttgtttgtttgaatttgaggccttgttataggtttcatatttctttctcttgttctaagtaaatg

tcagaataataatgtaatggggatcctctagagtcgag

SEQ ID NO: 6 *Arabidopsis* GS1 coding sequence
Cambia 1201 vector + rbcS3C + *arabidopsis* GS1Bold **ATG** is the start site,

AAAAAAGAAAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGG

ACGAGTGAGGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCAC

AAAATCCAATGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCG

TTAGATAGGAAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTA

ACCAATTATTTCAGCA***CCATG***TCTCTGCTCTCAGATCTCGTTAACCTCAACCTCA

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CCGATGCCACCGGGAAAATCATCGCCGAATACATATGGATCGGTGGATCTGGA

ATGGATATCAGAAGCAAAGCCAGGACACTACCAGGACCAGTGACTGATCCATCA

AAGCTTCCCAAGTGGAACTACGACGGATCCAGCACCGGTCAGGCTGCTGGAGA

AGACAGTGAAGTCATTCTATACCCTCAGGCAATATTCAAGGATCCCTTCAGGAAA

GGCAACAACATCCTGGTGATGTGTGATGCTTACACACCAGCTGGTGATCCTATT

CCAACCAACAAGAGGCACAACGCTGCTAAGATCTTCAGCCACCCCGACGTTGC

CAAGGAGGAGCCTTGGTATGGGATTGAGCAAGAATACACTTTGATGCAAAAGGA

TGTGAACTGGCCAATTGGTTGGCCTGTTGGTGGCTACCCTGGCCCTCAGGGAC

CTTACTACTGTGGTGTGGGAGCTGACAAAGCCATTGGTCGTGACATTGTGGATG

CTCACTACAAGGCCTGTCTTTACGCCGGTATTGGTATTTCTGGTATCAATGGAGA

AGTCATGCCAGGCCAGTGGGAGTTCCAAGTCGGCCCTGTTGAGGGTATTAGTT

CTGGTGATCAAGTCTGGGTTGCTGGATACCTTCTCGAGAGGATCACTGAGATCT

CTGGTGTAATTGTCAGCTTCGACCCGAAACCAGTCCCGGGTGACTGGAATGGA

GCTGGAGCTCACTGCAACTACAGCACTAAGACAATGAGAAACGATGGAGGATTA

GAAGTGATCAAGAAAGCGATAGGGAAGCTTCAGCTGAAACACAAAGAACACATT

GCTGCTTACGGTGAAGGAAACGAGCGTCGTCTCACTGGAAAGCACGAAACCGC

AGACATCAACACATTCTCTTGGGGAGTCGCGAACCGTGGAGCGTCAGTGAGAG

TGGGACGTGACACAGAGAAGGAAGGTAAAGGGTACTTCGAAGACAGAAGGCCA

GCTTCTAACATGGATCCTTACGTTGTCACCTCCATGATCGCTGAGACGACCATA

CTCGGTTGA

SEQ ID NO: 7 *Arabidopsis* GS1 amino acid sequence
Vector sequences at N-terminus in italics
*MVDLRNRRTS*MSLLSDLVNLNLTDATGKIIAEYIWIGGSGMDIRSKARTLPGPVTDPS

KLPKWNYDGSSTGQAAGEDSEVILYPQAIFKDPFRKGNNILVMCDAYTPAGDPIPTN

KRHNAAKIFSHPDVAKEEPWYGIEQEYTLMQKDVNWPIGWPVGGYPGPQGPYYC

GVGADKAIGRDIVDAHYKACLYAGIGISGINGEVMPGQWEFQVGPVEGISSGDQVW

VARYLLERITEISGVIVSFDPKPVPGDWNGAGAHCNYSTKTMRNDGGLEVIKKAIGK

LQLKHKEHIAAYGEGNERRLTGKHETADINTFSWGVANRGASVRVGRDTEKEGKG

YFEDRRPASNMDPYVVTSMIAETTILG

SEQ ID NO: 8 Grape GPT coding DNA sequence
Showing Cambia 1305.1 with (3' end of) rbcS3C + *Vitis vinifera* GPT (Grape). Bold **ATG** is the start site, parentheses are the catI intron and the underlined actagt is the speI cloning site used to splice in the GPT gene.
AAAAAAGAAAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGG

ACGAGTGAGGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCAC

AAAATCCAATGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCG

TTAGATAGGAAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTA

ACCAATTATTTCAGCA***CCATGG***TAGATCTGAGG(GTAAATTTCTAGTTTTTCTCCT

TCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTGAGCTTTGATCTTTCT

TTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAAATATTACATAGCTTTAA

CTGATAATCTGATTACTTTATTTCGTGTGTCTATGATGATGATGATAGTTACAG)A

ACCGACGA***ACTAGT***ATGCAGCTCTCTCAATGTACCTGGACATTCCCAGAGTTGC

-continued

TTAAAAGACCAGCCTTTTTAAGGAGGAGTATTGATAGTATTTCGAGTAGAAGTAG

GTCCAGCTCCAAGTATCCATCTTTCATGGCGTCCGCATCAACGGTCTCCGCTCC

AAATACGGAGGCTGAGCAGACCCATAACCCCCCTCAACCTCTACAGGTTGCAAA

GCGCTTGGAGAAATTCAAAACAACAATCTTTACTCAAATGAGCATGCTTGCCATC

AAACATGGAGCAATAAACCTTGGCCAAGGGTTTCCCAACTTTGATGGTCCTGAG

TTTGTCAAAGAAGCAGCAATTCAAGCCATTAAGGATGGGAAAAACCAATATGCTC

GTGGATATGGAGTTCCTGATCTCAACTCTGCTGTTGCTGATAGATTCAAGAAGG

ATACAGGACTCGTGGTGGACCCCGAGAAGGAAGTTACTGTTACTTCTGGATGTA

CAGAAGCAATTGCTGCTACTATGCTAGGCTTGATAAATCCTGGTGATGAGGTGA

TCCTCTTTGCTCCATTTTATGATTCCTATGAAGCCACTCTATCCATGGCTGGTGC

CCAAATAAAATCCATCACTTTACGTCCTCCGGATTTTGCTGTGCCCATGGATGAG

CTCAAGTCTGCAATCTCAAAGAATACCCGTGCAATCCTTATAAACACTCCCCATA

ACCCCACAGGAAAGATGTTCACAAGGGAGGAACTGAATGTGATTGCATCCCTCT

GCATTGAGAATGATGTGTTGGTGTTTACTGATGAAGTTTACGACAAGTTGGCTTT

CGAAATGGATCACATTTCCATGGCTTCTCTTCCTGGGATGTACGAGAGGACCGT

GACTATGAATTCCTTAGGGAAAACTTTCTCCCTGACTGGATGGAAGATTGGTTG

GACAGTAGCTCCCCCACACCTGACATGGGGAGTGAGGCAAGCCCACTCATTCC

TCACGTTTGCTACCTGCACCCCAATGCAATGGGCAGCTGCAACAGCCCTCCGG

GCCCCAGACTCTTACTATGAAGAGCTAAAGAGAGATTACAGTGCAAAGAAGGCA

ATCCTGGTGGAGGGATTGAAGGCTGTCGGTTTCAGGGTATACCCATCAAGTGG

GACCTATTTTGTGGTGGTGGATCACACCCCATTTGGGTTGAAAGACGATATTGC

GTTTTGTGAGTATCTGATCAAGGAAGTTGGGGTGGTAGCAATTCCGACAAGCGT

TTTCTACTTACACCCAGAAGATGGAAAGAACCTTGTGAGGTTTACCTTCTGTAAA

GACGAGGGAACTCTGAGAGCTGCAGTTGAAAGGATGAAGGAGAAACTGAAGCC

TAAACAATAGGGGCACGTGA

SEQ ID NO: 9 Grape GPT amino acid sequence
MVDLRNRRTSMQLSQCTWTFPELLKRPAFLRRSIDSISSRSRSSSKYPSFMASAST

VSAPNTEAEQTHNPPQPLQVAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGP

EFVKEAAIQAIKDGKNQYARGYGVPDLNSAVADRFKKDTGLVVDPEKEVTVTSGCT

EAIAATMLGLINPGDEVILFAPFYDSYEATLSMAGAQIKSITLRPPDFAVPMDELKSAI

SKNTRAILINTPHNPTGKMFTREELNVIASLCIENDVLVFTDEVYDKLAFEMDHISMAS

LPGMYERTVTMNSLGKTFSLTGWKIGWTVAPPHLTWGVRQAHSFLTFATCTPMQW

AAATALRAPDSYYEELKRDYSAKKAILVEGLKAVGFRVYPSSGTYFVVVDHTPFGLK

DDIAFCEYLIKEVGVVAIPTSVFYLHPEDGKNLVRFTFCKDEGTLRAAVERMKEKLKP

KQ

SEQ ID NO: 10 Rice GPT DNA coding sequence
Rice GPT codon optimized for *E. coli* expression; untranslated sequences shown in lower case
atgtggATGAACCTGGCAGGCTTTCTGGCAACCCCGGCAACCGCAACCGCAACCC

GTCATGAAATGCCGCTGAACCCGAGCAGCAGCGCGAGCTTTCTGCTGAGCAGC

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CTGCGTCGTAGCCTGGTGGCGAGCCTGCGTAAAGCGAGCCCGGCAGCAGCAG

CAGCACTGAGCCCGATGGCAAGCGCAAGCACCGTGGCAGCAGAAAACGGTGC

AGCAAAAGCAGCAGCAGAAAAACAGCAGCAGCAGCCGGTGCAGGTGGCGAAA

CGTCTGGAAAAATTTAAAACCACCATTTTTACCCAGATGAGCATGCTGGCGATTA

AACATGGCGCGATTAACCTGGGCCAGGGCTTTCC

GAACTTTGATGGCCCGGATTTTGTGAAAGAAGCGGCGATTCAGGCGATTAACGC

GGGCAAAAACCAGTATGCGCGTGGCTATGGCGTGCCGGAACTGAACAGCGCGA

TTGCGGAACGTTTTCTGAAAGATAGCGGCCTGCAGGTGGATCCGGAAAAAGAA

GTGACCGTGACCAGCGGCTGCACCGAAGCGATTGCGGCGACCATTCTGGGCCT

GATTAACCCGGGCGATGAAGTGATTCTGTTTGCGCCGTTTTATGATAGCTATGA

AGCGACCCTGAGCATGGCGGGCGCGAACGTGAAAGCGATTACCCTGCGTCCG

CCGGATTTTAGCGTGCCGCTGGAAGAACTGAAAGCGGCCGTGAGCAAAAACAC

CCGTGCGATTATGATTAACACCCCGCATAACCCGACCGGCAAAATGTTTACCCG

TGAAGAACTGGAATTTATTGCGACCCTGTGCAAAGAAAACGATGTGCTGCTGTT

TGCGGATGAAGTGTATGATAAACTGGCGTTTGAAGCGGATCATATTAGCATGGC

GAGCATTCCGGGCATGTATGAACGTACCGTGACCATGAACAGCCTGGGCAAAA

CCTTTAGCCTGACCGGCTGGAAAATTGGCTGGGCGATTGCGCCGCCGCATCTG

ACCTGGGGCGTGCGTCAGGCACATAGCTTTCTGACCTTTGCAACCTGCACCCC

GATGCAGGCAGCCGCCGCAGCAGCACTGCGTGCACCGGATAGCTATTATGAAG

AACTGCGTCGTGATTATGGCGCGAAAAAAGCGCTGCTGGTGAACGGCCTGAAA

GATGCGGGCTTTATTGTGTATCCGAGCAGCGGCACCTATTTTGTGATGGTGGAT

CATACCCCGTTTGGCTTTGATAACGATATTGAATTTTGCGAATATCTGATTCGTG

AAGTGGGCGTGGTGGCGATTCCGCCGAGCGTGTTTTATCTGAACCCGGAAGAT

GGCAAAAACCTGGTGCGTTTTACCTTTTGCAAAGATGATGAAACCCTGCGTGCG

GCGGTGGAACGTATGAAAACCAAACTGCGTAAAAAAAAGCTTgcggccgcactcgagc

accaccaccaccaccactga

SEQ ID NO: 11 Rice GPT amino acid sequence
Includes amino terminal amino acids *MW* for cloning and *His* tag sequences from pet28 vector in italics.
*MW*MNLAGFLATPATATATRHEMPLNPSSSASFLLSSLRRSLVASLRKASPAAAAAL

SPMASASTVAAENGAAKAAAEKQQQQPVQVAKRLEKFKTTIFTQMSMLAIKHGAINL

GQGFPNFDGPDFVKEAAIQAINAGKNQYARGYGVPELNSAIAERFLKDSGLQVDPE

KEVTVTSGCTEAIAATILGLINPGDEVILFAPFYDSYEATLSMAGANVKAITLRPPDFS

VPLEELKAAVSKNTRAIMINTPHNPTGKMFTREELEFIATLCKENDVLLFADEVYDKL

AFEADHISMASIPGMYERTVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAHSFL

TFATCTPMQAAAAAALRAPDSYYEELRRDYGAKKALLVNGLKDAGFIVYPSSGTYF

VMVDHTPFGFDNDIEFCEYLIREVGVVAIPPSVFYLNPEDGKNLVRFTFCKDDETLR

AAVERMKTKLRKK*KLAAALEHHHHHH*

-continued

SEQ ID NO: 12 Soybean GPT DNA coding sequence
TOPO 151D WITH SOYBEAN for *E. coli* expression
From starting codon. Vector sequences are italicized
ATG*CATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTC*

*GATTCTACGGAAAACCTGTATTTTCAGGGAATTGATCCCTTCACC*GCGAAACGT

CTGGAAAAATTTCAGACCACCATTTTTACCCAGATGAGCCTGCTGGCGATTAAAC

ATGGCGCGATTAACCTGGGCCAGGGCTTTCCGAACTTTGATGGCCCGGAATTT

GTGAAAGAAGCGGCGATTCAGGCGATTCGTGATGGCAAAAACCAGTATGCGCG

TGGCTATGGCGTGCCGGATCTGAACATTGCGATTGCGGAACGTTTTAAAAAAGA

TACCGGCCTGGTGGTGGATCCGGAAAAAGAAATTACCGTGACCAGCGGCTGCA

CCGAAGCGATTGCGGCGACCATGATTGGCCTGATTAACCCGGGCGATGAAGTG

ATTATGTTTGCGCCGTTTTATGATAGCTATGAAGCGACCCTGAGCATGGCGGGC

GCGAAAGTGAAAGGCATTACCCTGCGTCCGCCGGATTTTGCGGTGCCGCTGGA

AGAACTGAAAAGCACCATTAGCAAAAACACCCGTGCGATTCTGATTAACACCCC

GCATAACCCGACCGGCAAAATGTTTACCCGTGAAGAACTGAACTGCATTGCGAG

CCTGTGCATTGAAAACGATGTGCTGGTGTTTACCGATGAAGTGTATGATAAACT

GGCGTTTGATATGGAACATATTAGCATGGCGAGCCTGCCGGGCATGTTTGAACG

TACCGTGACCCTGAACAGCCTGGGCAAAACCTTTAGCCTGACCGGCTGGAAAAT

TGGCTGGGCGATTGCGCCGCCGCATCTGAGCTGGGGCGTGCGTCAGGCGCAT

GCGTTTCTGACCTTTGCAACCGCACATCCGTTTCAGTGCGCAGCAGCAGCAGCA

CTGCGTGCACCGGATAGCTATTATGTGGAACTGAAACGTGATTATATGGCGAAA

CGTGCGATTCTGATTGAAGGCCTGAAAGCGGTGGGCTTTAAAGTGTTTCCGAGC

AGCGGCACCTATTTTGTGGTGGTGGATCATACCCCGTTTGGCCTGGAAAACGAT

GTGGCGTTTTGCGAATATCTGGTGAAAGAAGTGGGCGTGGTGGCGATTCCGAC

CAGCGTGTTTTATCTGAACCCGGAAGAAGGCAAAAACCTGGTGCGTTTTACCTT

TTGCAAAGATGAAGAAACCATTCGTAGCGCGGTGGAACGTATGAAAGCGAAACT

GCGTAAAGTCGACTAA

SEQ ID NO: 13 Soybean GPT amino acid sequence
Translated protein product, vector sequences italicized
*MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFT*AKRLEKFQTTIFTQMSLLAIKHGAI

NLGQGFPNFDGPEFVKEAAIQAIRDGKNQYARGYGVPDLNIAIAERFKKDTGLVVDP

EKEITVTSGCTEAIAATMIGLINPGDEVIMFAPFYDSYEATLSMAGAKVKGITLRPPDF

AVPLEELKSTISKNTRAILINTPHNPTGKMFTREELNCIASLCIENDVLVFTDEVYDKL

AFDMEHISMASLPGMFERTVTLNSLGKTFSLTGWKIGWAIAPPHLSWGVRQAHAFL

TFATAHPFQCAAAAALRAPDSYYVELKRDYMAKRAILIEGLKAVGFKVFPSSGTYFV

VVDHTPFGLENDVAFCEYLVKEVGVVAIPTSVFYLNPEEGKNLVRFTFCKDEETIRS

AVERMKAKLRKVD

SEQ ID NO: 14 Barley GPT DNA coding sequence
Coding sequence from start with intron removed
ATGGTAGATCTGAGGAACCGACGA***ACTAGT***ATGGCATCCGCCCCCGCCTCCGC

CTCCGCGGCCCTCTCCACCGCCGCCCCCGCCGACAACGGGGCCGCCAAGCCC

ACGGAGCAGCGGCCGGTACAGGTGGCTAAGCGATTGGAGAAGTTCAAAACAAC

-continued

AATTTTCACACAGATGAGCATGCTCGCAGTGAAGCATGGAGCAATAAACCTTGG

ACAGGGGTTTCCCAATTTTGATGGCCCTGACTTTGTCAAAGATGCTGCTATTGA

GGCTATCAAAGCTGGAAAGAATCAGTATGCAAGAGGATATGGTGTGCCTGAATT

GAACTCAGCTGTTGCTGAGAGATTTCTCAAGGACAGTGGATTGCACATCGATCC

TGATAAGGAAGTTACTGTTACATCTGGGTGCACAGAAGCAATAGCTGCAACGAT

ATTGGGTCTGATCAACCCTGGGGATGAAGTCATACTGTTTGCTCCATTCTATGAT

TCTTATGAGGCTACACTGTCCATGGCTGGTGCGAATGTCAAAGCCATTACACTC

CGCCCTCCGGACTTTGCAGTCCCTCTTGAAGAGCTAAAGGCTGCAGTCTCGAA

GAATACCAGAGCAATAATGATTAATACACCTCACAACCCTACCGGGAAAATGTTC

ACAAGGGAGGAACTTGAGTTCATTGCTGATCTCTGCAAGGAAAATGACGTGTTG

CTCTTTGCCGATGAGGTCTACGACAAGCTGGCGTTTGAGGCGGATCACATATCA

ATGGCTTCTATTCCTGGCATGTATGAGAGGACCGTCACTATGAACTCCCTGGGG

AAGACGTTCTCCTTGACCGGATGGAAGATCGGCTGGGCGATAGCACCACCGCA

CCTGACATGGGGCGTAAGGCAGGCACACTCCTTCCTCACATTCGCCACCTCCA

CGCCGATGCAATCAGCAGCGGCGGCGGCCCTGAGAGCACCGGACAGCTACTT

TGAGGAGCTGAAGAGGGACTACGGCGCAAAGAAAGCGCTGCTGGTGGACGGG

CTCAAGGCGGCGGGCTTCATCGTCTACCCTTCGAGCGGAACCTACTTCATCATG

GTCGACCACACCCCGTTCGGGTTCGACAACGACGTCGAGTTCTGCGAGTACTT

GATCCGCGAGGTCGGCGTCGTGGCCATCCCGCCAAGCGTGTTCTACCTGAACC

CGGAGGACGGGAAGAACCTGGTGAGGTTCACCTTCTGCAAGGACGACGACACG

CTAAGGGCGGCGGTGGACAGGATGAAGGCCAAGCTCAGGAAGAAATGA

SEQ ID NO: 15 Barley GPT amino acid sequence
Translated sequence from start site (intron removed)
MVDLRNRRTSMASAPASASAALSTAAPADNGAAKPTEQRPVQVAKRLEKFKTTIFT

QMSMLAVKHGAINLGQGFPNFDGPDFVKDAAIEAIKAGKNQYARGYGVPELNSAVA

ERFLKDSGLHIDPDKEVTVTSGCTEAIAATILGLINPGDEVILFAPFYDSYEATLSMAG

ANVKAITLRPPDFAVPLEELKAAVSKNTRAIMINTPHNPTGKMFTREELEFIADLCKE

NDVLLFADEVYDKLAFEADHISMASIPGMYERTVTMNSLGKTFSLTGWKIGWAIAPP

HLTWGVRQAHSFLTFATSTPMQSAAAAALRAPDSYFEELKRDYGAKKALLVDGLKA

AGFIVYPSSGTYFIMVDHTPFGFDNDVEFCEYLIREVGVVAIPPSVFYLNPEDGKNLV

RFTFCKDDDTLRAAVDRMKAKLRKK

SEQ ID NO: 16 Zebra fish GPT DNA coding sequence
Danio rerio sequence designed for expression in *E coli*. Bold, italicized nucleotides added for cloning or from pET28b vector.
ATGTCCGTGGCGAAACGTCTGGAAAAATTTAAAACCACCATTTTTACCCAGATGA

GCATGCTGGCGATTAAACATGGCGCGATTAACCTGGGCCAGGGCTTTCCGAAC

TTTGATGGCCCGGATTTTGTGAAAGAAGCGGCGATTCAGGCGATTCGTGATGGC

AACAACCAGTATGCGCGTGGCTATGGCGTGCCGGATCTGAACATTGCGATTAG

CGAACGTTATAAAAAAGATACCGGCCTGGCGGTGGATCCGGAAAAAGAAATTAC

CGTGACCAGCGGCTGCACCGAAGCGATTGCGGCGACCGTGCTGGGCCTGATT

AACCCGGGCGATGAAGTGATTGTGTTTGCGCCGTTTTATGATAGCTATGAAGCG

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ACCCTGAGCATGGCGGGCGCGAAAGTGAAAGGCATTACCCTGCGTCCGCCGG

ATTTTGCGCTGCCGATTGAAGAACTGAAAAGCACCATTAGCAAAAACACCCGTG

CGATTCTGCTGAACACCCCGCATAACCCGACCGGCAAAATGTTTACCCCGGAAG

AACTGAACACCATTGCGAGCCTGTGCATTGAAAACGATGTGCTGGTGTTTAGCG

ATGAAGTGTATGATAAACTGGCGTTTGATATGGAACATATTAGCATTGCGAGCCT

GCCGGGCATGTTTGAACGTACCGTGACCATGAACAGCCTGGGCAAAACCTTTA

GCCTGACCGGCTGGAAAATTGGCTGGGCGATTGCGCCGCCGCATCTGACCTGG

GGCGTGCGTCAGGCGCATGCGTTTCTGACCTTTGCAACCAGCAACCCGATGCA

GTGGGCAGCAGCAGTGGCACTGCGTGCACCGGATAGCTATTATACCGAACTGA

AACGTGATTATATGGCGAAACGTAGCATTCTGGTGGAAGGCCTGAAAGCGGTG

GGCTTTAAAGTGTTTCCGAGCAGCGGCACCTATTTTGTGGTGGTGGATCATACC

CCGTTTGGCCATGAAAACGATATTGCGTTTTGCGAATATCTGGTGAAAGAAGTG

GGCGTGGTGGCGATTCCGACCAGCGTGTTTTATCTGAACCCGGAAGAAGGCAA

AAACCTGGTGCGTTTTACCTTTTGCAAAGATGAAGGCACCCTGCGTGCGGCGGT

GGATCGTATGAAAGAAAAACTGCGTAAA***GTCGACAAGCTTGCGGCCGCACTCG***

AGCACCACCACCACCACCACCACTGA

SEQ ID NO: 17 Zebra fish GPR amino acid sequence
Amino acid sequence of *Danio rerio* cloned and expressed in *E. coli* (bold, italicized amino acids are added from vector/cloning and His tag on C-terminus)
MSVAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPDFVKEAAIQAIRDGNNQ

YARGYGVPDLNIAISERYKKDTGLAVDPEKEITVTSGCTEAIAATVLGLINPGDEVIVF

APFYDSYEATLSMAGAKVKGITLRPPDFALPIEELKSTISKNTRAILLNTPHNPTGKMF

TPEELNTIASLCIENDVLVFSDEVYDKLAFDMEHISIASLPGMFERTVTMNSLGKTFSL

TGWKIGWAIAPPHLTWGVRQAHAFLTFATSNPMQWAAAVALRAPDSYYTELKRDY

MAKRSILVEGLKAVGFKVFPSSGTYFVVVDHTPFGHENDIAFCEYLVKEVGVVAIPT

SVFYLNPEEGKNLVRFTFCKDEGTLRAAVDRMKEKLRK***VDKLAAALEHHHHHH-***

SEQ ID NO: 18 *Arabidopsis* truncated GPT-30 construct DNA sequence
Arabidopsis GPT coding sequence with 30 amino acids removed from the targeting sequence.
ATGGCCAAAATCCATCGTCCTATCGGAGCCACCATGACCACAGTTTCGACTCAG

AACGAGTCTACTCAAAAACCCGTCCAGGTGGCGAAGAGATTAGAGAAGTTCAAG

ACTACTATTTTCACTCAAATGAGCATATTGGCAGTTAAACATGGAGCGATCAATT

TAGGCCAAGGCTTTCCCAATTTCGACGGTCCTGATTTTGTTAAAGAAGCTGCGA

TCCAAGCTATTAAAGATGGTAAAAACCAGTATGCTCGTGGATACGGCATTCCTCA

GCTCAACTCTGCTATAGCTGCGCGGTTTCGTGAAGATACGGGTCTTGTTGTTGA

TCCTGAGAAAGAAGTTACTGTTACATCTGGTTGCACAGAAGCCATAGCTGCAGC

TATGTTGGGTTTAATAAACCCTGGTGATGAAGTCATTCTCTTTGCACCGTTTTAT

GATTCCTATGAAGCAACACTCTCTATGGCTGGTGCTAAAGTAAAAGGAATCACTT

TACGTCCACCGGACTTCTCCATCCCTTTGGAAGAGCTTAAAGCTGCGGTAACTA

ACAAGACTCGAGCCATCCTTATGAACACTCCGCACAACCCGACCGGGAAGATGT

TCACTAGGGAGGAGCTTGAAACCATTGCATCTCTCTGCATTGAAAACGATGTGC

TTGTGTTCTCGGATGAAGTATACGATAAGCTTGCGTTTGAAATGGATCACATTTC

-continued

TATAGCTTCTCTTCCCGGTATGTATGAAAGAACTGTGACCATGAATTCCCTGGGA

AAGACTTTCTCTTTAACCGGATGGAAGATCGGCTGGGCGATTGCGCCGCCTCAT

CTGACTTGGGGAGTTCGACAAGCACACTCTTACCTCACATTCGCCACATCAACA

CCAGCACAATGGGCAGCCGTTGCAGCTCTCAAGGCACCAGAGTCTTACTTCAAA

GAGCTGAAAAGAGATTACAATGTGAAAAAGGAGACTCTGGTTAAGGGTTTGAAG

GAAGTCGGATTTACAGTGTTCCCATCGAGCGGGACTTACTTTGTGGTTGCTGAT

CACACTCCATTTGGAATGGAGAACGATGTTGCTTTCTGTGAGTATCTTATTGAAG

AAGTTGGGGTCGTTGCGATCCCAACGAGCGTCTTTTATCTGAATCCAGAAGAAG

GGAAGAATTTGGTTAGGTTTGCGTTCTGTAAAGACGAAGAGACGTTGC

GTGGTGCAATTGAGAGGATGAAGCAGAAGCTTAAGAGAAAAGTCTGA

SEQ ID NO: 19 *Arabidopsis* truncated GPT-30 construct amino acid sequence
MAKIHRPIGATMTTVSTQNESTQKPVQVAKRLEKFKTTIFTQMSILAVKHGAINLGQG

FPNFDGPDFVKEAAIQAIKDGKNQYARGYGIPQLNSAIAARFREDTGLVVDPEKEVT

VTSGCTEAIAAAMLGLINPGDEVILFAPFYDSYEATLSMAGAKVKGITLRPPDFSIPLE

ELKAAVTNKTRAILMNTPHNPTGKMFTREELETIASLCIENDVLVFSDEVYDKLAFEM

DHISIASLPGMYERTVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAHSYLTFATS

TPAQWAAVAALKAPESYFKELKRDYNVKKETLVKGLKEVGFTVFPSSGTYFVVADH

TPFGMENDVAFCEYLIEEVGVVAIPTSVFYLNPEEGKNLVRFAFCKDEETLRGAIER

MKQKLKRKV

SEQ ID NO: 20: *Arabidopsis* truncated GPT-45 construct DNA sequence
Arabidopsis GPT coding sequence with 45 residues in the targeting sequence removed
ATGGCGACTCAGAACGAGTCTACTCAAAAACCCGTCCAGGTGGCGAAGAGATTA

GAGAAGTTCAAGACTACTATTTTCACTCAAATGAGCATATTGGCAGTTAAACATG

GAGCGATCAATTTAGGCCAAGGCTTTCCCAATTTCGACGGTCCTGATTTTGTTAA

AGAAGCTGCGATCCAAGCTATTAAAGATGGTAAAAACCAGTATGCTCGTGGATA

CGGCATTCCTCAGCTCAACTCTGCTATAGCTGCGCGGTTTCGTGAAGATACGGG

TCTTGTTGTTGATCCTGAGAAAGAAGTTACTGTTACATCTGGTTGCACAGAAGCC

ATAGCTGCAGCTATGTTGGGTTTAATAAACCCTGGTGATGAAGTCATTCTCTTTG

CACCGTTTTATGATTCCTATGAAGCAACACTCTCTATGGCTGGTGCTAAAGTAAA

AGGAATCACTTTACGTCCACCGGACTTCTCCATCCCTTTGGAAGAGCTTAAAGC

TGCGGTAACTAACAAGACTCGAGCCATCCTTATGAACACTCCGCACAACCCGAC

CGGGAAGATGTTCACTAGGGAGGAGCTTGAAACCATTGCATCTCTCTGCATTGA

AAACGATGTGCTTGTGTTCTCGGATGAAGTATACGATAAGCTTGCGTTTGAAATG

GATCACATTTCTATAGCTTCTCTTCCCGGTATGTATGAAAGAACTGTGACCATGA

ATTCCCTGGGAAAGACTTTCTCTTTAACCGGATGGAAGATCGGCTGGGCGATTG

CGCCGCCTCATCTGACTTGGGGAGTTCGACAAGCACACTCTTACCTCACATTCG

CCACATCAACACCAGCACAATGGGCAGCCGTTGCAGCTCTCAAGGCACCAGAG

TCTTACTTCAAAGAGCTGAAAAGAGATTACAATGTGAAAAAGGAGACTCTGGTTA

AGGGTTTGAAGGAAGTCGGATTTACAGTGTTCCCATCGAGCGGGACTTACTTTG

-continued

TGGTTGCTGATCACACTCCATTTGGAATGGAGAACGATGTTGCTTTCTGTGAGTA

TCTTATTGAAGAAGTTGGGGTCGTTGCGATCCCAACGAGCGTCTTTTATCTGAAT

CCAGAAGAAGGGAAGAATTTGGTTAGGTTTGCGTTCTGTAAAGACGAAGAGACG

TTGCGTGGTGCAATTGAGAGGATGAAGCAGAAGCTTAAGAGAAAAGTCTGA

SEQ ID NO: 21: *Arabidopsis* truncated GPT-45 construct amino acid sequence
MATQNESTQKPVQVAKRLEKFKTTIFTQMSILAVKHGAINLGQGFPNFDGPDFVKEA

AIQAIKDGKNQYARGYGIPQLNSAIAARFREDTGLVVDPEKEVTVTSGCTEAIAAAML

GLINPGDEVILFAPFYDSYEATLSMAGAKVKGITLRPPDFSIPLEELKAAVTNKTRAIL

MNTPHNPTGKMFTREELETIASLCIENDVLVFSDEVYDKLAFEMDHISIASLPGMYER

TVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAHSYLTFATSTPAQWAAVAALKA

PESYFKELKRDYNVKKETLVKGLKEVGFTVFPSSGTYFVVADHTPFGMENDVAFCE

YLIEEVGVVAIPTSVFYLNPEEGKNLVRFAFCKDEETLRGAIERMKQKLKRKV

SEQ ID NO: 22: Tomato Rubisco promoter
TOMATO RuBisCo rbcS3C promoter sequence from KpnI to NcoI
*GGTACC*GTTTGAATCCTCCTTAAAGTTTTTCTCTGGAGAAACTGTAGTAATTTTAC

TTTGTTGTGTTCCCTTCATCTTTTGAATTAATGGCATTTGTTTTAATACTAATCTGC

TTCTGAAACTTGTAATGTATGTATATCAGTTTCTTATAATTTATCCAAGTAATATCT

TCCATTCTCTATGCAATTGCCTGCATAAGCTCGACAAAAGAGTACATCAACCCCT

CCTCCTCTGGACTACTCTAGCTAAACTTGAATTTCCCCTTAAGATTATGAAATTG

ATATATCCTTAACAAACGACTCCTTCTGTTGGAAAATGTAGTACTTGTCTTTCTTC

TTTTGGGTATATATAGTTTATATACACCATACTATGTACAACATCCAAGTAGAGTG

AAATGGATACATGTACAAGACTTATTTGATTGATTGATGACTTGAGTTGCCTTAG

GAGTAACAAATTCTTAGGTCAATAAATCGTTGATTTGAAATTAATCTCTCTGTCTT

AGACAGATAGGAATTATGACTTCCAATGGTCCAGAAAGCAAAGTTCGCACTGAG

GGTATACTTGGAATTGAGACTTGCACAGGTCCAGAAACCAAAGTTCCCATCGAG

CTCTAAAATCACATCTTTGGAATGAAATTCAATTAGAGATAAGTTGCTTCATAGCA

TAGGTAAAATGGAAGATGTGAAGTAACCTGCAATAATCAGTGAAATGACATTAAT

ACACTAAATACTTCATATGTAATTATCCTTTCCAGGTTAACAATACTCTATAAAGT

AAGAATTATCAGAAATGGGCTCATCAAACTTTTGTACTATGTATTTCATATAAGGA

AGTATAACTATACATAAGTGTATACACAACTTTATTCCTATTTTGTAAAGGTGGAG

AGACTGTTTTCGATGGATCTAAAGCAATATGTCTATAAAATGCATTGATATAATAA

TTATCTGAGAAAATCCAGAATTGGCGTTGGATTATTTCAGCCAAATAGAAGTTTG

TACCATACTTGTTGATTCCTTCTAAGTTAAGGTGAAGTATCATTCATAAACAGTTT

TCCCCAAAGTACTACTCACCAAGTTTCCCTTTGTAGAATTAACAGTTCAAATATAT

GGCGCAGAAATTACTCTATGCCCAAAACCAAACGAGAAAGAAACAAAATACAGG

GGTTGCAGACTTTATTTTCGTGTTAGGGTGTGTTTTTTCATGTAATTAATCAAAAA

ATATTATGACAAAAACATTTATACATATTTTTACTCAACACTCTGGGTATCAGGGT

GGGTTGTGTTCGACAATCAATATGGAAAGGAAGTATTTTCCTTATTTTTTTAGTTA

ATATTTTCAGTTATACCAAACATACCTTGTGATATTATTTTTAAAAATGAAAAACTC

GTCAGAAAGAAAAAGCAAAAGCAACAAAAAAATTGCAAGTATTTTTTAAAAAAGA

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AAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGGACGAGTGA

GGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCACAAAATCCAA

TGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCGTTAGATAGG

AAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTAACCAATTATT

TCAGCA*CC*ATG*G*

SEQ ID NO: 23: Bamboo GPT DNA coding sequence
ATGGCCTCCGCGGCCGTCTCCACCGTCGCCACCGCCGCCGACGGCGTCGCGA

AGCCGACGGAGAAGCAGCCGGTACAGGTCGCAAAGCGTTTGGAAAAGTTTAAG

ACAACAATTTTCACACAGATGAGCATGCTTGCCATCAAGCATGGAGCAATAAAC

CTCGGCCAGGGCTTTCCGAATTTTGATGGCCCTGACTTTGTGAAAGAAGCTGCT

ATTCAAGCTATCAATGCTGGGAAGAATCAGTATGCAAGAGGATATGGTGTGCCT

GAACTGAACTCGGCTGTTGCTGAAAGGTTCCTGAAGGACAGTGGCTTGCAAGTC

GATCCCGAGAAGGAAGTTACTGTCACATCTGGGTGCACGGAAGCGATAGCTGC

AACGATATTGGGTCTTATCAACCCTGGCGATGAAGTGATCTTGTTTGCTCCATTC

TATGATTCATACGAGGCTACGCTGTCGATGGCTGGTGCCAATGTAAAAGCCATT

ACTCTCCGTCCTCCAGATTTTGCAGTCCCTCTTGAGGAGCTAAAGGCCACAGTC

TCTAAGAACACCAGAGCGATAATGATAAACACACCACACAATCCTACTGGGAAA

ATGTTTTCTAGGGAAGAACTTGAATTCATTGCTACTCTCTGCAAGAAAAATGATG

TGTTGCTTTTTGCTGATGAGGTCTATGACAAGTTGGCATTTGAGGCAGATCATAT

ATCAATGGCTTCTATTCCTGGCATGTATGAGAGGACTGTGACTATGAACTCTCTG

GGGAAGACATTCTCTCTAACAGGATGGAAGATCGGTTGGGCAATAGCACCACCA

CACCTGACATGGGGTGTAAGGCAGGCACACTCATTCCTCACATTTGCCACCTGC

ACACCAATGCAATCGGCGGCGGCGGCGGCTCTTAGAGCACCAGATAGCTACTA

TGGGGAGCTGAAGAGGGATTACGGTGCAAAGAAAGCGATACTAGTCGACGGAC

TCAAGGCTGCAGGTTTTATTGTTTACCCTTCAAGTGGAACATACTTTGTCATGGT

CGATCACACCCCGTTTGGTTTCGACAATGATATTGAGTTCTGCGAGTATTTGATC

CGCGAAGTCGGTGTTGTCGCCATACCACCAAGCGTATTTTATCTCAACCCTGAG

GATGGGAAGAACTTGGTGAGGTTCACCTTCTGCAAGGATGATGATACGCTGAGA

GCCGCAGTTGAGAGGATGAAGACAAAGCTCAGGAAAAAATGA

SEQ ID NO: 24: Bamboo GPT amino acid sequence
MASAAVSTVATAADGVAKPTEKQPVQVAKRLEKFKTTIFTQMSMLAIKHGAINLGQG

FPNFDGPDFVKEAAIQAINAGKNQYARGYGVPELNSAVAERFLKDSGLQVDPEKEV

TVTSGCTEAIAATILGLINPGDEVILFAPFYDSYEATLSMAGANVKAITLRPPDFAVPL

EELKATVSKNTRAIMINTPHNPTGKMFSREELEFIATLCKKNDVLLFADEVYDKLAFE

ADHISMASIPGMYERTVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAHSFLTFA

TCTPMQSAAAAALRAPDSYYGELKRDYGAKKAILVDGLKAAGFIVYPSSGTYFVMV

DHTPFGFDNDIEFCEYLIREVGVVAIPPSVFYLNPEDGKNLVRFTFCKDDDTLRAAVE

RMKTKLRKK

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SEQ ID NO: 25: 1305.1 + rbcS3C promoter + catI intron with rice GPT gene. Cambia1305.1 with (3' end of) rbcS3C + rice GPT coding sequence. Underlined ATG is start site, parentheses are the catI intron and the underlined actagt is the speI cloning site used to splice in the rice gene.

AAAAAAGAAAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGG

ACGAGTGAGGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCAC

AAAATCCAATGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCG

TTAGATAGGAAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTA

ACCAATTATTTCAGCA***CCATGG***TAGATCTGAGG(GTAAATTTCTAGTTTTTCTCCT

TCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTGAGCTTTGATCTTTCT

TTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAAATATTACATAGCTTTAA

CTGATAATCTGATTACTTTATTTCGTGTGTCTATGATGATGATGATAGTTACAG)A

ACCGACGA***ACTAGT***ATGAATCTGGCCGGCTTTCTCGCCACGCCCGCGACCGCG

ACCGCGACGCGGCATGAGATGCCGTTAAATCCCTCCTCCTCCGCCTCCTTCCTC

CTCTCCTCGCTCCGCCGCTCGCTCGTCGCGTCGCTCCGGAAGGCCTCGCCGG

CGGCGGCCGCGGCGCTCTCCCCCATGGCCTCCGCGTCCACCGTCGCCGCCGA

GAACGGCGCCGCCAAGGCGGCGGCGGAGAAGCAGCAGCAGCAGCCTGTGCA

GGTTGCAAAGCGGTTGGAAAAGTTTAAGACGACCATTTTCACACAGATGAGTAT

GCTTGCCATCAAGCATGGAGCAATAAACCTTGGCCAGGGTTTTCCGAATTTCGA

TGGCCCTGACTTTGTAAAAGAGGCTGCTATTCAAGCTATCAATGCTGGGAAGAA

TCAGTACGCAAGAGGATATGGTGTGCCTGAACTGAACTCAGCTATTGCTGAAAG

ATTCCTGAAGGACAGCGGACTGCAAGTCGATCCGGAGAAGGAAGTTACTGTCA

CATCTGGATGCACAGAAGCTATAGCTGCAACAATTTTAGGTCTAATTAATCCAGG

CGATGAAGTGATATTGTTTGCTCCATTCTATGATTCATATGAGGCTACCCTGTCA

ATGGCTGGTGCCAACGTAAAAGCCATTACTCTCCGTCCTCCAGATTTTTCAGTC

CCTCTTGAAGAGCTAAAGGCTGCAGTCTCGAAGAACACCAGAGCTATTATGATA

AACACCCCGCACAATCCTACTGGGAAAATGTTTACAAGGGAAGAACTTGAGTTT

ATTGCCACTCTCTGCAAGGAAAATGATGTGCTGCTTTTTGCTGATGAGGTCTAC

GACAAGTTAGCTTTTGAGGCAGATCATATATCAATGGCTTCTATTCCTGGCATGT

ATGAGAGGACCGTGACCATGAACTCTCTTGGGAAGACATTCTCTCTTACAGGAT

GGAAGATCGGTTGGGCAATCGCACCGCCACACCTGACATGGGGTGTAAGGCAG

GCACACTCATTCCTCACGTTTGCGACCTGCACACCAATGCAAGCAGCTGCAGCT

GCAGCTCTGAGAGCACCAGATAGCTACTATGAGGAACTGAGGAGGGATTATGG

AGCTAAGAAGGCATTGCTAGTCAACGGACTCAAGGATGCAGGTTTCATTGTCTA

TCCTTCAAGTGGAACATACTTCGTCATGGTCGACCACACCCCATTTGGTTTCGA

CAATGATATTGAGTTCTGCGAGTATTTGATTCGCGAAGTCGGTGTTGTCGCCATA

CCACCTAGTGTATTTTATCTCAACCCTGAGGATGGGAAGAACTTGGTGAGGTTC

ACCTTTTGCAAGGATGATGAGACGCTGAGAGCCGCGGTTGAGAGGATGAAGAC

AAAGCTCAGGAAAAAATGA

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SEQ ID NO: 26: *HORDEUM* GPT SEQUENCE IN VECTOR
Cambia1305.1 with (3' end of) rbcS3C + *hordeum* (IDI4) coding sequence. Underlined **ATG** is start site, parentheses are the catI intron and the underlined actagt is the speI cloning site used to splice in the *hordeum* gene.

AAAAAAGAAAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGG

ACGAGTGAGGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCAC

AAAATCCAATGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCG

TTAGATAGGAAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTA

ACCAATTATTTCAGCACC***CCATGG***TAGATCTGAGG(GTAAATTTCTAGTTTTTCTCCT

TCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTGAGCTTTGATCTTTCT

TTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAAATATTACATAGCTTTAA

CTGATAATCTGATTACTTTATTTCGTGTGTCTATGATGATGATGATAGTTACAG)A

ACCGACGA***ACTAGT***ATGGCATCCGCCCCCGCCTCCGCCTCCGCGGCCCTCTCC

ACCGCCGCCCCCGCCGACAACGGGGCCGCCAAGCCCACGGAGCAGCGGCCG

GTACAGGTGGCTAAGCGATTGGAGAAGTTCAAAACAACAATTTTCACACAGATG

AGCATGCTCGCAGTGAAGCATGGAGCAATAAACCTTGGACAGGGGTTTCCCAAT

TTTGATGGCCCTGACTTTGTCAAAGATGCTGCTATTGAGGCTATCAAAGCTGGA

AAGAATCAGTATGCAAGAGGATATGGTGTGCCTGAATTGAACTCAGCTGTTGCT

GAGAGATTTCTCAAGGACAGTGGATTGCACATCGATCCTGATAAGGAAGTTACT

GTTACATCTGGGTGCACAGAAGCAATAGCTGCAACGATATTGGGTCTGATCAAC

CCTGGGGATGAAGTCATACTGTTTGCTCCATTCTATGATTCTTATGAGGCTACAC

TGTCCATGGCTGGTGCGAATGTCAAAGCCATTACACTCCGCCCTCCGGACTTTG

CAGTCCCTCTTGAAGAGCTAAAGGCTGCAGTCTCGAAGAATACCAGAGCAATAA

TGATTAATACACCTCACAACCCTACCGGGAAAATGTTCACAAGGGAGGAACTTG

AGTTCATTGCTGATCTCTGCAAGGAAAATGACGTGTTGCTCTTTGCCGATGAGG

TCTACGACAAGCTGGCGTTTGAGGCGGATCACATATCAATGGCTTCTATTCCTG

GCATGTATGAGAGGACCGTCACTATGAACTCCCTGGGGAAGACGTTCTCCTTGA

CCGGATGGAAGATCGGCTGGGCGATAGCACCACCGCACCTGACATGGGGCGT

AAGGCAGGCACACTCCTTCCTCACATTCGCCACCTCCACGCCGATGCAATCAGC

AGCGGCGGCGGCCCTGAGAGCACCGGACAGCTACTTTGAGGAGCTGAAGAGG

GACTACGGCGCAAAGAAAGCGCTGCTGGTGGACGGGCTCAAGGCGGCGGGCT

TCATCGTCTACCCTTCGAGCGGAACCTACTTCATCATGGTCGACCACACCCCGT

TCGGGTTCGACAACGACGTCGAGTTCTGCGAGTACTTGATCCGCGAGGTCGGC

GTCGTGGCCATCCCGCCAAGCGTGTTCTACCTGAACCCGGAGGACGGGAAGAA

CCTGGTGAGGTTCACCTTCTGCAAGGACGACGACACGCTAAGGGCGGCGGTG

GACAGGATGAAGGCCAAGCTCAGGAAGAAATGATTGAGGGGCG***CACGTGTGA***

SEQ ID NO: 27 Expression cassette, *Arabidopsis* GPT coding sequence (ATG underlined) under control of CMV 35S promoter (italics; promoter from Cambia 1201)

CATGGAGTCAAAGATTCAAATAGAGGACCTAACAGAACTCGCCGTAAAGACTGG

CGAACAGTTCATACAGAGTCTCTTACGACTCAATGACAAGAAGAAAATCTTCGTC

AACATGGTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCT

CAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACC

-continued

TCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAA

GGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGA

AGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCA

TCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTG

ATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACC

CTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGAACACGGGGGACTCTTGA

*CC*ATGTACCTGGACATAAATGGTGTGATGATCAAACAGTTTAGCTTCAAAGCCTC

TCTTCTCCCATTCTCTTCTAATTTCCGACAAAGCTCCGCCAAAATCCATCGTCCT

ATCGGAGCCACCATGACCACAGTTTCGACTCAGAACGAGTCTACTCAAAAACCC

GTCCAGGTGGCGAAGAGATTAGAGAAGTTCAAGACTACTATTTTCACTCAAATG

AGCATATTGGCAGTTAAACATGGAGCGATCAATTTAGGCCAAGGCTTTCCCAATT

TCGACGGTCCTGATTTTGTTAAAGAAGCTGCGATCCAAGCTATTAAAGATGGTAA

AAACCAGTATGCTCGTGGATACGGCATTCCTCAGCTCAACTCTGCTATAGCTGC

GCGGTTTCGTGAAGATACGGGTCTTGTTGTTGATCCTGAGAAAGAAGTTACTGT

TACATCTGGTTGCACAGAAGCCATAGCTGCAGCTATGTTGGGTTTAATAAACCCT

GGTGATGAAGTCATTCTCTTTGCACCGTTTTATGATTCCTATGAAGCAACACTCT

CTATGGCTGGTGCTAAAGTAAAAGGAATCACTTTACGTCCACCGGACTTCTCCA

TCCCTTTGGAAGAGCTTAAAGCTGCGGTAACTAACAAGACTCGAGCCATCCTTA

TGAACACTCCGCACAACCCGACCGGGAAGATGTTCACTAGGGAGGAGCTTGAA

ACCATTGCATCTCTCTGCATTGAAAACGATGTGCTTGTGTTCTCGGATGAAGTAT

ACGATAAGCTTGCGTTTGAAATGGATCACATTTCTATAGCTTCTCTTCCCGGTAT

GTATGAAAGAACTGTGACCATGAATTCCCTGGGAAAGACTTTCTCTTTAACCGGA

TGGAAGATCGGCTGGGCGATTGCGCCGCCTCATCTGACTTGGGGAGTTCGACA

AGCACACTCTTACCTCACATTCGCCACATCAACACCAGCACAATGGGCAGCCGT

TGCAGCTCTCAAGGCACCAGAGTCTTACTTCAAAGAGCTGAAAAGAGATTACAA

TGTGAAAAAGGAGACTCTGGTTAAGGGTTTGAAGGAAGTCGGATTTACAGTGTT

CCCATCGAGCGGGACTTACTTTGTGGTTGCTGATCACACTCCATTTGGAATGGA

GAACGATGTTGCTTTCTGTGAGTATCTTATTGAAGAAGTTGGGGTCGTTGCGAT

CCCAACGAGCGTCTTTTATCTGAATCCAGAAGAAGGGAAGAATTTGGTTAGGTT

TGCGTTCTGTAAAGACGAAGAGACGTTGCGTGGTGCAATTGAGAGGATGAAGC

AGAAGCTTAAGAGAAAAGTCTGA

SEQ ID NO: 28 Cambia p1305.1 with (3' end of) rbcS3C + *Arabidopsis* GPT coding sequence. Underlined ATG is start site, parentheses are the catI intron and the underlined actagt is the speI cloning site used to splice in the *Arabidopsis* gene.

AAAAAAGAAAAAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGG

ACGAGTGAGGGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCAC

AAAATCCAATGGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCG

TTAGATAGGAAGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTA

ACCAATTATTTCAGCA***CCATGG***TAGATCTGAGG(GTAAATTTCTAGTTTTTCTCCT

TCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTGAGCTTTGATCTTTCT

-continued

TTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAAATATTACATAGCTTTAA

CTGATAATCTGATTACTTTATTTCGTGTGTCTATGATGATGATGATAGTTACAG)A

ACCGACGA***ACTAGT***ATGTACCTGGACATAAATGGTGTGATGATCAAACAGTTTA

GCTTCAAAGCCTCTCTTCTCCCATTCTCTTCTAATTTCCGACAAAGCTCCGCCAA

AATCCATCGTCCTATCGGAGCCACCATGACCACAGTTTCGACTCAGAACGAGTC

TACTCAAAAACCCGTCCAGGTGGCGAAGAGATTAGAGAAGTTCAAGACTACTAT

TTTCACTCAAATGAGCATATTGGCAGTTAAACATGGAGCGATCAATTTAGGCCAA

GGCTTTCCCAATTTCGACGGTCCTGATTTTGTTAAAGAAGCTGCGATCCAAGCTA

TTAAAGATGGTAAAAACCAGTATGCTCGTGGATACGGCATTCCTCAGCTCAACT

CTGCTATAGCTGCGCGGTTTCGTGAAGATACGGGTCTTGTTGTTGATCCTGAGA

AAGAAGTTACTGTTACATCTGGTTGCACAGAAGCCATAGCTGCAGCTATGTTGG

GTTTAATAAACCCTGGTGATGAAGTCATTCTCTTTGCACCGTTTTATGATTCCTAT

GAAGCAACACTCTCTATGGCTGGTGCTAAAGTAAAAGGAATCACTTTACGTCCA

CCGGACTTCTCCATCCCTTTGGAAGAGCTTAAAGCTGCGGTAACTAACAAGACT

CGAGCCATCCTTATGAACACTCCGCACAACCCGACCGGGAAGATGTTCACTAG

GGAGGAGCTTGAAACCATTGCATCTCTCTGCATTGAAAACGATGTGCTTGTGTT

CTCGGATGAAGTATACGATAAGCTTGCGTTTGAAATGGATCACATTTCTATAGCT

TCTCTTCCCGGTATGTATGAAAGAACTGTGACCATGAATTCCCTGGGAAAGACTT

TCTCTTTAACCGGATGGAAGATCGGCTGGGCGATTGCGCCGCCTCATCTGACTT

GGGGAGTTCGACAAGCACACTCTTACCTCACATTCGCCACATCAACACCAGCAC

AATGGGCAGCCGTTGCAGCTCTCAAGGCACCAGAGTCTTACTTCAAAGAGCTGA

AAAGAGATTACAATGTGAAAAAGGAGACTCTGGTTAAGGGTTTGAAGGAAGTCG

GATTTACAGTGTTCCCATCGAGCGGGACTTACTTTGTGGTTGCTGATCACACTC

CATTTGGAATGGAGAACGATGTTGCTTTCTGTGAGTATCTTATTGAAGAAGTTGG

GGTCGTTGCGATCCCAACGAGCGTCTTTTATCTGAATCCAGAAGAAGGGAAGAA

TTTGGTTAGGTTTGCGTTCTGTAAAGACGAAGAGACGTTGCGTGGTGCAATTGA

GAGGATGAAGCAGAAGCTTAAGAGAAAAGTCTGA

SEQ ID NO: 29 *Arabidpsis* GPT coding sequence (mature protein, no targeting sequence)

GTGGCGAAGAGATTAGAGAAGTTCAAGACTACTATTTTCACTCAAATGAGCATAT

TGGCAGTTAAACATGGAGCGATCAATTTAGGCCAAGGCTTTCCCAATTTCGACG

GTCCTGATTTTGTTAAAGAAGCTGCGATCCAAGCTATTAAAGATGGTAAAAACCA

GTATGCTCGTGGATACGGCATTCCTCAGCTCAACTCTGCTATAGCTGCGCGGTT

TCGTGAAGATACGGGTCTTGTTGTTGATCCTGAGAAAGAAGTTACTGTTACATCT

GGTTGCACAGAAGCCATAGCTGCAGCTATGTTGGGTTTAATAAACCCTGGTGAT

GAAGTCATTCTCTTTGCACCGTTTTATGATTCCTATGAAGCAACACTCTCTATGG

CTGGTGCTAAAGTAAAAGGAATCACTTTACGTCCACCGGACTTCTCCATCCCTTT

GGAAGAGCTTAAAGCTGCGGTAACTAACAAGACTCGAGCCATCCTTATGAACAC

TCCGCACAACCCGACCGGGAAGATGTTCACTAGGGAGGAGCTTGAAACCATTG

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CATCTCTCTGCATTGAAAACGATGTGCTTGTGTTCTCGGATGAAGTATACGATAA

GCTTGCGTTTGAAATGGATCACATTTCTATAGCTTCTCTTCCCGGTATGTATGAA

AGAACTGTGACCATGAATTCCCTGGGAAAGACTTTCTCTTTAACCGGATGGAAG

ATCGGCTGGGCGATTGCGCCGCCTCATCTGACTTGGGGAGTTCGACAAGCACA

CTCTTACCTCACATTCGCCACATCAACACCAGCACAATGGGCAGCCGTTGCAGC

TCTCAAGGCACCAGAGTCTTACTTCAAAGAGCTGAAAAGAGATTACAATGTGAAA

AAGGAGACTCTGGTTAAGGGTTTGAAGGAAGTCGGATTTACAGTGTTCCCATCG

AGCGGGACTTACTTTGTGGTTGCTGATCACACTCCATTTGGAATGGAGAACGAT

GTTGCTTTCTGTGAGTATCTTATTGAAGAAGTTGGGGTCGTTGCGATCCCAACG

AGCGTCTTTTATCTGAATCCAGAAGAAGGGAAGAATTTGGTTAGGTTTGCGTTCT

GTAAAGACGAAGAGACGTTGCGTGGTGCAATTGAGAGGATGAAGCAGAAGCTT

AAGAGAAAAGTCTGA

SEQ ID NO: 30 *Arabidpsis* GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSILAVKHGAINLGQGFPNFDGPDFVKEAAIQAIKDGKNQYAR

GYGIPQLNSAIAARFREDTGLVVDPEKEVTVTSGCTEAIAAAMLGLINPGDEVILFAP

FYDSYEATLSMAGAKVKGITLRPPDFSIPLEELKAAVTNKTRAILMNTPHNPTGKMFT

REELETIASLCIENDVLVFSDEVYDKLAFEMDHISIASLPGMYERTVTMNSLGKTFSL

TGWKIGWAIAPPHLTWGVRQAHSYLTFATSTPAQWAAVAALKAPESYFKELKRDYN

VKKETLVKGLKEVGFTVFPSSGTYFVVADHTPFGMENDVAFCEYLIEEVGVVAIPTS

VFYLNPEEGKNLVRFAFCKDEETLRGAIERMKQKLKRKV

SEQ ID NO: 31 Grape GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPEFVKEAAIQAIKDGKNQYAR

GYGVPDLNSAVADRFKKDTGLVVDPEKEVTVTSGCTEAIAATMLGLINPGDEVILFA

PFYDSYEATLSMAGAQIKSITLRPPDFAVPMDELKSAISKNTRAILINTPHNPTGKMFT

REELNVIASLCIENDVLVFTDEVYDKLAFEMDHISMASLPGMYERTVTMNSLGKTFS

LTGWKIGWTVAPPHLTWGVRQAHSFLTFATCTPMQWAAATALRAPDSYYEELKRD

YSAKKAILVEGLKAVGFRVYPSSGTYFVVVDHTPFGLKDDIAFCEYLIKEVGVVAIPT

SVFYLHPEDGKNLVRFTFCKDEGTLRAAVERMKEKLKPKQ

SEQ ID NO: 32 Rice GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPDFVKEAAIQAINAGKNQYAR

GYGVPELNSAIAERFLKDSGLQVDPEKEVTVTSGCTEAIAATILGLINPGDEVILFAPF

YDSYEATLSMAGANVKAITLRPPDFSVPLEELKAAVSKNTRAIMINTPHNPTGKMFT

REELEFIATLCKENDVLLFADEVYDKLAFEADHISMASIPGMYERTVTMNSLGKTFSL

TGWKIGWAIAPPHLTWGVRQAHSFLTFATCTPMQAAAAAALRAPDSYYEELRRDY

GAKKALLVNGLKDAGFIVYPSSGTYFVMVDHTPFGFDNDIEFCEYLIREVGVVAIPPS

VFYLNPEDGKNLVRFTFCKDDETLRAAVERMKTKLRKK

SEQ ID NO: 33 Soybean GPT amino acid sequence (-1 mature protein, no targeting sequence)
AKRLEKFQTTIFTQMSLLAIKHGAINLGQGFPNFDGPEFVKEAAIQAIRDGKNQYARG

YGVPDLNIAIAERFKKDTGLVVDPEKEITVTSGCTEAIAATMIGLINPGDEVIMFAPFY

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DSYEATLSMAGAKVKGITLRPPDFAVPLEELKSTISKNTRAILINTPHNPTGKMFTRE

ELNCIASLCIENDVLVFTDEVYDKLAFDMEHISMASLPGMFERTVTLNSLGKTFSLTG

WKIGWAIAPPHLSWGVRQAHAFLTFATAHPFQCAAAAALRAPDSYYVELKRDYMAK

RAILIEGLKAVGFKVFPSSGTYFVVVDHTPFGLENDVAFCEYLVKEVGVVAIPTSVFY

LNPEEGKNLVRFTFCKDEETIRSAVERMKAKLRKVD

SEQ ID NO: 34 Barley GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSMLAVKHGAINLGQGFPNFDGPDFVKDAAIEAIKAGKNQYA

RGYGVPELNSAVAERFLKDSGLHIDPDKEVTVTSGCTEAIAATILGLINPGDEVILFAP

FYDSYEATLSMAGANVKAITLRPPDFAVPLEELKAAVSKNTRAIMINTPHNPTGKMFT

REELEFIADLCKENDVLLFADEVYDKLAFEADHISMASIPGMYERTVTMNSLGKTFSL

TGWKIGWAIAPPHLTWGVRQAHSFLTFATSTPMQSAAAAALRAPDSYFEELKRDYG

AKKALLVDGLKAAGFIVYPSSGTYFIMVDHTPFGFDNDVEFCEYLIREVGVVAIPPSV

FYLNPEDGKNLVRFTFCKDDDTLRAAVDRMKAKLRKK

SEQ ID NO: 35 Zebra fish GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPDFVKEAAIQAIRDGNNQYA

RGYGVPDLNIAISERYKKDTGLAVDPEKEITVTSGCTEAIAATVLGLINPGDEVIVFAP

FYDSYEATLSMAGAKVKGITLRPPDFALPIEELKSTISKNTRAILLNTPHNPTGKMFTP

EELNTIASLCIENDVLVFSDEVYDKLAFDMEHISIASLPGMFERTVTMNSLGKTFSLT

GWKIGWAIAPPHLTWGVRQAHAFLTFATSNPMQWAAAVALRAPDSYYTELKRDYM

AKRSILVEGLKAVGFKVFPSSGTYFVVVDHTPFGHENDIAFCEYLVKEVGVVAIPTSV

FYLNPEEGKNLVRFTFCKDEGTLRAAVDRMKEKLRK

SEQ ID NO: 36 Bamboo GPT amino acid sequence (mature protein, no targeting sequence)
VAKRLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPDFVKEAAIQAINAGKNQYAR

GYGVPELNSAVAERFLKDSGLQVDPEKEVTVTSGCTEAIAATILGLINPGDEVILFAP

FYDSYEATLSMAGANVKAITLRPPDFAVPLEELKATVSKNTRAIMINTPHNPTGKMFS

REELEFIATLCKKNDVLLFADEVYDKLAFEADHISMASIPGMYERTVTMNSLGKTFSL

TGWKIGWAIAPPHLTWGVRQAHSFLTFATCTPMQSAAAAALRAPDSYYGELKRDY

GAKKAILVDGLKAAGFIVYPSSGTYFVMVDHTPFGFDNDIEFCEYLIREVGVVAIPPS

VFYLNPEDGKNLVRFTFCKDDDTLRAAVERMKTKLRKK

SEQ ID NO: 39 Rice rubisco promoter deposited in NCBI GenBank: AF143510.1
PstI cloning sites in bold; NcoI cloning site in italics, catI intron and part of Gus plus protein from Cambia 1305.1 vector in bold underline (sequence removed and not translated), 3' terminal SpeI cloning site in double underline. The construct also includes a PmlI 1305.1 cloning site CACGTG (also cuts in rice rbsc promoter), and a ZraI cloning site GACGTC, which can be added by PCR to clone into PmlI site of vector).
CTGCAGCAAAGAAACGTTATTAGTTGGTGCTTTTGGTGGTAGGAATGTAGTTTTC

TGACAAAGTCAATTACTGAATATAAAAAAAATCTGCACAGCTCTGCGTCAACAGT

TGTCCAAGGGATGCCTCAAAAATCTGTGCAGATTATCAGTCGTCACGCAGAAGC

AGAACATCATGGTGTGCTAGGTCAGCTTCTTGCATTGGGCCATGAATCCGGTTG

GTTGTTAATCTCTCCTCTCTTATTCTCTTATATTAAGATGCATAACTCTTTTATGTA

GTCTAAAAAAAAATCCAGTGGATCGGATAGTAGTACGTCATGGTGCCATTAGGT

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ACCGTTGAACCTAACAGATATTTATGCATGTGTATATATATAGCTATATAGACAAA

ATTGATGCCGATTATAGACCCAAAAGCAATAGGTATATATAATATAATACAGACC

ACACCACCAAACTAAGAATCGATCAAATAGACAAGGCATGTCTCCAAATTGTCTT

AAACTATTTCCGTAGGTTCAGCCGTTCAGGAGTCGAATCAGCCTCTGCCGGCGT

TTTCTTTGCACGTACGACGGACACACATGGGCATACCATATAGCTGGTCCATGA

CATTAGGAGAGAGAACGTACGTGTTGACCTGTAGCTGAGATATAACAAGGTTGA

TTATAATATCACCAAACATGAAATCATCCAAGGATGACCCATAACTATCACTACTA

TAGTACTGCATCTGGTAAAAGAAATTGTATAGACTCTATTTCGAGCACTACCACA

TAACGCCTGCAATGTGACACCCTACCTATTCACTAATGTGCCTCTTCCCACACG

CTTTCCACCCGTACTGCTCACAGCTTTAAGAACCAGAACAAATGAGTAATATTAG

TGTCGGTTCATGGCTAAAACCAGCACTGATGTACATGACCACATATGTCAAATG

CTGCTTCTAGGCATGACCCGCTCTTACTAATACCTACTCATCGCTAGAAGAATTT

TCGGCTGATAAATTTTCAATTTAAGCAAGAGTTATCTGCGTTGGTTCATAACTCA

AACTGATGGCCCCAACCATATTAGTGCAAATTTCACATATGATCATAACCTTTTC

ATATGAAATCGGATCGAGATGAACTTTATATAAACATTGTAGCTGTCGATGATAC

CTACAATTTTATAGTTCACAACCTTTTTATTTCAAGTCATTTAAATGCCCAAATAG

GTGTTTCAAATCTCAGATAGAAATGTTCAAAAGTAAAAAAGGTCCCTATCATAAC

ATAATTGATATGTAAGTGAGTTGGAAAAAGATAAGTACGTGTGAGAGAGATCGG

GGATCAAATTCTGGTGTAATAATGTATGTATTTCAGTCATAAAAATTGGTAGCAG

TAGTTGGGGCTCTGTATATATACCGGTAAGGATGGGATGGTAGTAGAATAATTC

TTTTTTTGTTTTTAGTTTTTTCTGGTCCAAAATTTCAAATTTGGATCCCTTACTTGT

ACCAACTAATATTAATGAGTGTTGAGGGTAGTAGAGGTGCAACTTTACCATAATC

CCTCTGTTTCAGGTTATAAGACGTTTTGACTTTAAATTTGACCAAGTTTATGCGCA

AATATAGTAATATTTATAATACTATATTAGTTTCATTAAATAAATAATTGAATATATT

TTCATAATAAATTTGTGTTGAGTTCAAAATATTATTAATTTTTTCTACAAACTTGGT

CAAACTTGAAGCAGTTTGACTTTGACCAAAGTCAAAACGTCTTATAACTTGAAAC

GGATGGATTACTTTTTTTGTGGGGACAAGTTTACAATGTTTAATAAAGCACAATC

CATCTTAATGTTTTCAAGCTGAATATTGTAAAATTCATGGATAAACCAGCTTCTAA

ATGTTTAACCGGGAAAATGTCGAACGACAAATTAATATTTTTAAGTGATGGGGAG

TATTAATTAAGGAGTGACAACTCAACTTTCAATATCGTACTAAACTGTGGGATTTA

TTTTCTAAAATTTTATACCCTGCCAATTCACGTGTTGTAGATCTTTTTTTTTCACTA

ACCGACACCAGGTATATCAATTTTATTGAATATAGCAGCAAAAAGAATGTGTTGT

ACTTGTAAACAAAAAGCAAACTGTACATAAAAAAAAATGCACTCCTATATAATTAA

GCTCATAAAGATGCTTTGCTTCGTGAGGGCCCAAGTTTTGATGACCTTTTGCTTG

ATCTCGAAATTAAAATTTAAGTACTGTTAAGGGAGTTCACACCACCATCAATTTTC

AGCCTGAAGAAACAGTTAAACAACGACCCCGATGACCAGTCTACTGCTCTCCAC

ATACTAGCTGCATTATTGATCACAAAACAAAACAAAACGAAATAAAAATCAGCAG

CGAGAGTGTGCAGAGAGAGACAAAGGTGATCTGGCGTGGATATCTCCCCATCC

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ATCCTCACCCGCGCTGCCCATCACTCGCCGCCGCATACTCCATCATGTGGAGA

GAGGAAGACGAGGACCACAGCCAGAGCCCGGGTCGAGATGCCACCACGGCCA

CAACCCACGAGCCCGGCGCGACACCACCGCGCGCGCGTGAGCCAGCCACAAA

CGCCCGCGGATAGGCGCGCGCACGCCGGCCAATCCTACCACATCCCCGGCCT

CCGCGGCTCGCGAGCGCCGCTGCCATCCGATCCGCTGAGTTTTGGCTATTTAT

ACGTACCGCGGGAGCCTGTGTGCAGAGCAGTGCATCTCAAGAAGTACTCGAGC

AAAGAAGGAGAGAGCTTGGTGAG**CTGCAG**CC*ATG*GTAGATCTGAGG**GTAAATT**

TCTAGTTTTTCTCCTTCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTG

AGCTTTGATCTTTCTTTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAA

ATATTACATAGCTTTAACTGATAATCTGATTACTTTATTTCGTGTGTCTATGATG

ATGATGATAGTTACAGAACCGACGAACTAGT

SEQ ID NO: 40 Horeum GS1 coding sequence
GCGCAGGCGGTTGTGCAGGCGATGCAGTGCCAGGTGGGGGTGAGGGGCAGG

ACGGCCGTCCCGGCGAGGCAGCCCGCGGGCAGGGTGTGGGGCGTCAGGAGG

GCCGCCCGCGCCACCTCCGGGTTCAAGGTGCTGGCGCTCGGCCCGGAGACCA

CCGGGGTCATCCAGAGGATGCAGCAGCTGCTCGACATGGACACCACGCCCTTC

ACCGACAAGATCATCGCCGAGTACATCTGGGTTGGAGGATCTGGAATTGACCTC

AGAAGCAAATCAAGGACGATTTCGAAGCCAGTGGAGGACCCGTCAGAGCTGCC

GAAATGGAACTACGACGGATCGAGCACGGGGCAGGCTCCTGGGGAAGACAGT

GAAGTCATCCTATACCCACAGGCCATATTCAAGGACCCATTCCGAGGAGGCAAC

AACATACTGGTTATCTGTGACACCTACACACCACAGGGGGAACCCATCCCTACT

AACAAACGCCACATGGCTGCACAAATCTTCAGTGACCCCAAGGTCACTTCACAA

GTGCCATGGTTCGGAATCGAACAGGAGTACACTCTGATGCAGAGGGATGTGAA

CTGGCCTCTTGGCTGGCCTGTTGGAGGGTACCCTGGCCCCCAGGGTCCATACT

ACTGCGCCGTAGGATCAGACAAGTCATTTGGCCGTGACATATCAGATGCTCACT

ACAAGGCGTGCCTTTACGCTGGAATTGAAATCAGTGGAACAAACGGGGAGGTC

ATGCCTGGTCAGTGGGAGTACCAGGTTGGACCCAGCGTTGGTATTGATGCAGG

AGACCACATATGGGCTTCCAGATACATTCTCGAGAGAATCACGGAGCAAGCTGG

TGTGGTGCTCACCCTTGACCCAAAACCAATCCAGGGTGACTGGAACGGAGCTG

GCTGCCACACAAACTACAGCACATTGAGCATGCGCGAGGATGGAGGTTTCGAC

GTGATCAAGAAGGCAATCCTGAACCTTTCACTTCGCCATGACTTGCACATAGCC

GCATATGGTGAAGGAAACGAGCGGAGGTTGACAGGGCTACACGAGACAGCTAG

CATATCAGACTTCTCATGGGGTGTGGCGAACCGTGGCTGCTCTATTCGTGTGGG

GCGAGACACCGAGGCGAAGGGCAAAGGATACCTGGAGGACCGTCGCCCGGCC

TCCAACATGGACCCGTACACCGTGACGGCGCTGCTGGCCGAGACCACGATCCT

GTGGGAGCCGACCCTCGAGGCGGAGGCCCTCGCTGCCAAGAAGCTGGCGCTG

AAGGTATGA

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SEQ ID NO: 41 Horeum GS1 amino acid sequence
AQAVVQAMQCQVGVRGRTAVPARQPAGRVWGVRRAARATSGFKVLALGPETTGV

IQRMQQLLDMDTTPFTDKIIAEYIWVGGSGIDLRSKSRTISKPVEDPSELPKWNYDG

SSTGQAPGEDSEVILYPQAIFKDPFRGGNNILVICDTYTPQGEPIPTNKRHMAAQIFS

DPKVTSQVPWFGIEQEYTLMQRDVNWPLGWPVGGYPGPQGPYYCAVGSDKSFG

RDISDAHYKACLYAGIEISGTNGEVMPGQWEYQVGPSVGIDAGDHIWASRYILERIT

EQAGVVLTLDPKPIQGDWNGAGCHTNYSTLSMREDGGFDVIKKAILNLSLRHDLHIA

AYGEGNERRLTGLHETASISDFSWGVANRGCSIRVGRDTEAKGKGYLEDRRPASN

MDPYTVTALLAETTILWEPTLEAEALAAKKLALKV

SEQ ID NO: 42: Expression cassette combining SEQ ID NO: 39 (5') and SEQ ID NO: 40 (3'), encoding the Rice rubisco promoter, catI intron and part of Gus plus protein, and hordeum GS1. Features shown as in SEQ ID NO: 39. *Hordeum* GS1 coding sequence begins after SpeI cloning site (double underline).
CTGCAGCAAAGAAACGTTATTAGTTGGTGCTTTTGGTGGTAGGAATGTAGTTTTC

TGACAAAGTCAATTACTGAATATAAAAAAAATCTGCACAGCTCTGCGTCAACAGT

TGTCCAAGGGATGCCTCAAAAATCTGTGCAGATTATCAGTCGTCACGCAGAAGC

AGAACATCATGGTGTGCTAGGTCAGCTTCTTGCATTGGGCCATGAATCCGGTTG

GTTGTTAATCTCTCCTCTCTTATTCTCTTATATTAAGATGCATAACTCTTTTATGTA

GTCTAAAAAAAAATCCAGTGGATCGGATAGTAGTACGTCATGGTGCCATTAGGT

ACCGTTGAACCTAACAGATATTTATGCATGTGTATATATATAGCTATATAGACAAA

ATTGATGCCGATTATAGACCCAAAAGCAATAGGTATATATAATATAATACAGACC

ACACCACCAAACTAAGAATCGATCAAATAGACAAGGCATGTCTCCAAATTGTCTT

AAACTATTTCCGTAGGTTCAGCCGTTCAGGAGTCGAATCAGCCTCTGCCGGCGT

TTTCTTTGCACGTACGACGGACACACATGGGCATACCATATAGCTGGTCCATGA

CATTAGGAGAGAGAACGTACGTGTTGACCTGTAGCTGAGATATAACAAGGTTGA

TTATAATATCACCAAACATGAAATCATCCAAGGATGACCCATAACTATCACTACTA

TAGTACTGCATCTGGTAAAAGAAATTGTATAGACTCTATTTCGAGCACTACCACA

TAACGCCTGCAATGTGACACCCTACCTATTCACTAATGTGCCTCTTCCCACACG

CTTTCCACCCGTACTGCTCACAGCTTTAAGAACCAGAACAAATGAGTAATATTAG

TGTCGGTTCATGGCTAAAACCAGCACTGATGTACATGACCACATATGTCAAATG

CTGCTTCTAGGCATGACCCGCTCTTACTAATACCTACTCATCGCTAGAAGAATTT

TCGGCTGATAAATTTTCAATTTAAGCAAGAGTTATCTGCGTTGGTTCATAACTCA

AACTGATGGCCCCAACCATATTAGTGCAAATTTCACATATGATCATAACCTTTTC

ATATGAAATCGGATCGAGATGAACTTTATATAAACATTGTAGCTGTCGATGATAC

CTACAATTTTATAGTTCACAACCTTTTTATTTCAAGTCATTTAAATGCCCAAATAG

GTGTTTCAAATCTCAGATAGAAATGTTCAAAAGTAAAAAAGGTCCCTATCATAAC

ATAATTGATATGTAAGTGAGTTGGAAAAAGATAAGTACGTGTGAGAGAGATCGG

GGATCAAATTCTGGTGTAATAATGTATGTATTTCAGTCATAAAAATTGGTAGCAG

TAGTTGGGGCTCTGTATATATACCGGTAAGGATGGGATGGTAGTAGAATAATTC

TTTTTTTGTTTTTAGTTTTTTCTGGTCCAAAATTTCAAATTTGGATCCCTTACTTGT

ACCAACTAATATTAATGAGTGTTGAGGGTAGTAGAGGTGCAACTTTACCATAATC

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CCTCTGTTTCAGGTTATAAGACGTTTTGACTTTAAATTTGACCAAGTTTATGCGCA

AATATAGTAATATTTATAATACTATATTAGTTTCATTAAATAAATAATTGAATATATT

TTCATAATAAATTTGTGTTGAGTTCAAAATATTATTAATTTTTTCTACAAACTTGGT

CAAACTTGAAGCAGTTTGACTTTGACCAAAGTCAAAACGTCTTATAACTTGAAAC

GGATGGATTACTTTTTTGTGGGGACAAGTTTACAATGTTTAATAAAGCACAATC

CATCTTAATGTTTTCAAGCTGAATATTGTAAAATTCATGGATAAACCAGCTTCTAA

ATGTTTAACCGGGAAAATGTCGAACGACAAATTAATATTTTTAAAGTGATGGGGAG

TATTAATTAAGGAGTGACAACTCAACTTTCAATATCGTACTAAACTGTGGGATTTA

TTTTCTAAAATTTTATACCCTGCCAATTCACGTGTTGTAGATCTTTTTTTTTCACTA

ACCGACACCAGGTATATCAATTTTATTGAATATAGCAGCAAAAAGAATGTGTTGT

ACTTGTAAACAAAAAGCAAACTGTACATAAAAAAAAATGCACTCCTATATAATTAA

GCTCATAAAGATGCTTTGCTTCGTGAGGGCCCAAGTTTTGATGACCTTTTGCTTG

ATCTCGAAATTAAAATTTAAGTACTGTTAAGGGAGTTCACACCACCATCAATTTTC

AGCCTGAAGAAACAGTTAAACAACGACCCCGATGACCAGTCTACTGCTCTCCAC

ATACTAGCTGCATTATTGATCACAAAACAAAACAAAACGAAATAAAAATCAGCAG

CGAGAGTGTGCAGAGAGAGACAAAGGTGATCTGGCGTGGATATCTCCCCATCC

ATCCTCACCCGCGCTGCCCATCACTCGCCGCCGCATACTCCATCATGTGGAGA

GAGGAAGACGAGGACCACAGCCAGAGCCCGGGTCGAGATGCCACCACGGCCA

CAACCCACGAGCCCGGCGCGACACCACCGCGCGCGCGTGAGCCAGCCACAAA

CGCCCGCGGATAGGCGCGCGCACGCCGGCCAATCCTACCACATCCCCGGCCT

CCGCGGCTCGCGAGCGCCGCTGCCATCCGATCCGCTGAGTTTTGGCTATTTAT

ACGTACCGCGGGAGCCTGTGTGCAGAGCAGTGCATCTCAAGAAGTACTCGAGC

AAAGAAGGAGAGAGCTTGGTGAGCTGCAG*CC*ATG*G*TAGATCTGAGGGTAAATTT

CTAGTTTTTCTCCTTCATTTTCTTGGTTAGGACCCTTTTCTCTTTTTATTTTTTTGA

GCTTTGATCTTTCTTTAAACTGATCTATTTTTTAATTGATTGGTTATGGTGTAAATA

TTACATAGCTTTAACTGATAATCTGATTACTTTATTTCGTGTGTCTATGATGATGA

TGATAGTTACAGAACCGACGAACTAGTGCGCAGGCGGTTGTGCAGGCGATGCA

GTGCCAGGTGGGGGTGAGGGGCAGGACGGCCGTCCCGGCGAGGCAGCCCGC

GGGCAGGGTGTGGGGCGTCAGGAGGGCCGCCCGCGCCACCTCCGGGTTCAA

GGTGCTGGCGCTCGGCCCGGAGACCACCGGGGTCATCCAGAGGATGCAGCAG

CTGCTCGACATGGACACCACGCCCTTCACCGACAAGATCATCGCCGAGTACATC

TGGGTTGGAGGATCTGGAATTGACCTCAGAAGCAAATCAAGGACGATTTCGAAG

CCAGTGGAGGACCCGTCAGAGCTGCCGAAATGGAACTACGACGGATCGAGCAC

GGGGCAGGCTCCTGGGGAAGACAGTGAAGTCATCCTATACCCACAGGCCATAT

TCAAGGACCCATTCCGAGGAGGCAACAACATACTGGTTATCTGTGACACCTACA

CACCACAGGGGGAACCCATCCCTACTAACAAACGCCACATGGCTGCACAAATCT

TCAGTGACCCCAAGGTCACTTCACAAGTGCCATGGTTCGGAATCGAACAGGAGT

ACACTCTGATGCAGAGGGATGTGAACTGGCCTCTTGGCTGGCCTGTTGGAGGG

TACCCTGGCCCCCAGGGTCCATACTACTGCGCCGTAGGATCAGACAAGTCATTT

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GGCCGTGACATATCAGATGCTCACTACAAGGCGTGCCTTTACGCTGGAATTGAA

ATCAGTGGAACAAACGGGGAGGTCATGCCTGGTCAGTGGGAGTACCAGGTTGG

ACCCAGCGTTGGTATTGATGCAGGAGACCACATATGGGCTTCCAGATACATTCT

CGAGAGAATCACGGAGCAAGCTGGTGTGGTGCTCACCCTTGACCCAAAACCAA

TCCAGGGTGACTGGAACGGAGCTGGCTGCCACACAAACTACAGCACATTGAGC

ATGCGCGAGGATGGAGGTTTCGACGTGATCAAGAAGGCAATCCTGAACCTTTCA

CTTCGCCATGACTTGCACATAGCCGCATATGGTGAAGGAAACGAGCGGAGGTT

GACAGGGCTACACGAGACAGCTAGCATATCAGACTTCTCATGGGGTGTGGCGA

ACCGTGGCTGCTCTATTCGTGTGGGGCGAGACACCGAGGCGAAGGGCAAAGG

ATACCTGGAGGACCGTCGCCCGGCCTCCAACATGGACCCGTACACCGTGACGG

CGCTGCTGGCCGAGACCACGATCCTGTGGGAGCCGACCCTCGAGGCGGAGGC

CCTCGCTGCCAAGAAGCTGGCGCTGAAGGTATGA

SEQ ID NO: 43 Amino acid sequence of translation product of SEQ ID NO: 42.
Amino-terminal bold residues from Gusplus and SpeI cloning site (intron removed)
MVDLRNRRTSAQAVVQAMQCQVGVRGRTAVPARQPAGRVWGVRRAARATSGFK

VLALGPETTGVIQRMQQLLDMDTTPFTDKIIAEYIWVGGSGIDLRSKSRTISKPVEDP

SELPKWNYDGSSTGQAPGEDSEVILYPQAIFKDPFRGGNNILVICDTYTPQGEPIPT

NKRHMAAQIFSDPKVTSQVPWFGIEQEYTLMQRDVNWPLGWPVGGYPGPQGPYY

CAVGSDKSFGRDISDAHYKACLYAGIEISGTNGEVMPGQWEYQVGPSVGIDAGDHI

WASRYILERITEQAGVVLTLDPKPIQGDWNGAGCHTNYSTLSMREDGGFDVIKKAIL

NLSLRHDLHIAAYGEGNERRLTGLHETASISDFSWGVANRGCSIRVGRDTEAKGKG

YLEDRRPASNMDPYTVTALLAETTILWEPTLEAEALAAKKLALKV

SEQ ID NO: 44 Maize ubil promoter: 5'UTR intron shown in italics, TATA box at -30
is underlined, 5' and 3' PstI cloning sites in bold
CTGCAGTGCAGCGTGACCCGGTCGTGCCCCTCTCTAGAGATAATGAGCATTGC

ATGTCTAAGTTATAAAAAATTACCACATATTTTTTTGTCACACTTGTTTGAAGTG

CAGTTTATCTATCTTTATACATATATTTAAACTTTACTCTACGAATAATATAATCTA

TAGTACTACAATAATATCAGTGTTTTAGAGAATCATATAAATGAACAGTTAGACAT

GGTCTAAAGGACAATTGAGTATTTTGACAACAGGACTCTACAGTTTTATCTTTTTA

GTGTGCATGTGTTCTCCTTTTTTTTTGCAAATAGCTTCACCTATATAATACTTCAT

CCATTTTATTAGTACATCCATTTAGGGTTTAGGGTTAATGGTTTTTATAGACTAAT

TTTTTTAGTACATCTATTTTATTCTATTTTAGCCTCTAAATTAAGAAAACTAAAACT

CTATTTTAGTTTTTTTATTTAATAATTTAGATATAAAATAGAATAAAATAAAGTGAC

TAAAAATTAAACAAATACCCTTTAAGAAATTAAAAAAACTAAGGAAACATTTTTCTT

GTTTCGAGTAGATAATGCCAGCCTGTTAAACGCCGTCGACGAGTCTAACGGACA

CCAACCAGCGAACCAGCAGCGTCGCGTCGGGCCAAGCGAAGCAGACGGCACG

GCATCTCTGTCGCTGCCTCTGGACCCCTCTCGAGAGTTCCGCTCCACCGTTGG

ACTTGCTCCGCTGTCGGCATCCAGAAATTGCGTGGCGGAGCGGCAGACGTGAG

CCGGCACGGCAGGCGGCCTCCTCCTCCTCTCACGGCACGGCAGCTACGGGGG

ATTCCTTTCCCACCGCTCCTTCGCTTTCCCTTCCTCGCCCGCCGTAATAAATAGA

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CACCCCCTCCACACCCTCTTTCCCCAACCTCGTGTTGTTCGGAGCGCACACACA

CACAACCAGATCTCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTACG

CCGCTCGTCCTCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGT

CCATGGTTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGT

TTGTGTTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACGTCAG

ACACGTTCTGATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCCTGGGATGG

CTCTAGCCGTTCCGCAGACGGGATCGATTTCATGATTTTTTTTGTTTCGTTGCAT

AGGGTTTGGTTTGCCCTTTTCCTTTATTTCAATATATGCCGTGCACTTGTTTGTC

GGGTCATCTTTTCATGCTTTTTTTTGTCTTGGTTGTGATGATGTGGTCTGGTTGG

GCGGTCGTTCTAGATCGGAGTAGAATTCTGTTTCAAACTACCTGGTGGATTTATT

AATTTTGGATCTGTATGTGTGTGCCATACATATTCATAGTTACGAATTGAAGATG

ATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATGCGGGTTTTACTGA

TGCATATACAGAGATGCTTTTTGTTCGCTTGGTTGTGATGATGTGGTGTGGTTGG

GCGGTCGTTCATTCGTTCTAGATCGGAGTAGAATACTGTTTCAAACTACCTGGT

GTATTTATTAATTTTGGAACTGTATGTGTGTGTCATACATCTTCATAGTTACGAGT

TTAAGATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATGTGGGTTTT

ACTGATGCATATACATGATGGCATATGCAGCATCTATTCATATGCTCTAACCTTG

AGTACCTATCTATTATAATAAACAAGTATGTTTTATAATTATTTTGATCTTGATATA

CTTGGATGATGGCATATGCAGCAGCTATATGTGGATTTTTTTAGCCCTGCCTTCA

TACGCTATTTATTTGCTTGGTACTGTTTCTTTTGTCGATGCTCACCCTGTTGTTTG

*GTGTTACTT***CTGCAG**

SEQ ID NO: 45 *Hordeum* GPT DNA coding sequence, including targeting sequence coding domain

ATGGCATCCGCCCCCGCCTCCGCCTCCGCGGCCCTCTCCACCGCCGCCCCCG

CCGACAACGGGGCCGCCAAGCCCACGGAGCAGCGGCCGGTACAGGTGGCTAA

GCGATTGGAGAAGTTCAAAACAACAATTTTCACACAGATGAGCATGCTCGCAGT

GAAGCATGGAGCAATAAACCTTGGACAGGGGTTTCCCAATTTTGATGGCCCTGA

CTTTGTCAAAGATGCTGCTATTGAGGCTATCAAAGCTGGAAAGAATCAGTATGCA

AGAGGATATGGTGTGCCTGAATTGAACTCAGCTGTTGCTGAGAGATTTCTCAAG

GACAGTGGATTGCACATCGATCCTGATAAGGAAGTTACTGTTACATCTGGGTGC

ACAGAAGCAATAGCTGCAACGATATTGGGTCTGATCAACCCTGGGGATGAAGTC

ATACTGTTTGCTCCATTCTATGATTCTTATGAGGCTACACTGTCCATGGCTGGTG

CGAATGTCAAAGCCATTACACTCCGCCCTCCGGACTTTGCAGTCCCTCTTGAAG

AGCTAAAGGCTGCAGTCTCGAAGAATACCAGAGCAATAATGATTAATACACCTC

ACAACCCTACCGGGAAAATGTTCACAAGGGAGGAACTTGAGTTCATTGCTGATC

TCTGCAAGGAAAATGACGTGTTGCTCTTTGCCGATGAGGTCTACGACAAGCTGG

CGTTTGAGGCGGATCACATATCAATGGCTTCTATTCCTGGCATGTATGAGAGGA

CCGTCACTATGAACTCCCTGGGGAAGACGTTCTCCTTGACCGGATGGAAGATC

GGCTGGGCGATAGCACCACCGCACCTGACATGGGGCGTAAGGCAGGCACACT

CCTTCCTCACATTCGCCACCTCCACGCCGATGCAATCAGCAGCGGCGGCGGCC

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CTGAGAGCACCGGACAGCTACTTTGAGGAGCTGAAGAGGGACTACGGCGCAAA

GAAAGCGCTGCTGGTGGACGGGCTCAAGGCGGCGGGCTTCATCGTCTACCCTT

CGAGCGGAACCTACTTCATCATGGTCGACCACACCCCGTTCGGGTTCGACAAC

GACGTCGAGTTCTGCGAGTACTTGATCCGCGAGGTCGGCGTCGTGGCCATCCC

GCCAAGCGTGTTCTACCTGAACCCGGAGGACGGGAAGAACCTGGTGAGGTTCA

CCTTCTGCAAGGACGACGACACGCTAAGGGCGGCGGTGGACAGGATGAAGGC

CAAGCTCAGGAAGAAATGA

SEQ ID NO: 46: *Hordeum* GPT amino acid sequence, including putative targeting sequence (in italics).

*MASAPASASAALSTAAPADNGAAKPTEQRPVQV*AKRLEKFKTTIFTQMSMLAVKHG

AINLGQGFPNFDGPDFVKDAAIEAIKAGKNQYARGYGVPELNSAVAERFLKDSGLHI

DPDKEVTVTSGCTEAIAATILGLINPGDEVILFAPFYDSYEATLSMAGANVKAITLRPP

DFAVPLEELKAAVSKNTRAIMINTPHNPTGKMFTREELEFIADLCKENDVLLFADEVY

DKLAFEADHISMASIPGMYERTVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAH

SFLTFATSTPMQSAAAAALRAPDSYFEELKRDYGAKKALLVDGLKAAGFIVYPSSGT

YFIMVDHTPFGFDNDVEFCEYLIREVGVVAIPPSVFYLNPEDGKNLVRFTFCKDDDT

LRAAVDRMKAKLRKK

SEQ ID NO: 47 Tomato rubisco small subunit (rbcS3C) promoter + *Arabidopsis* GS1 DNA coding sequence; NcoI/AflIII splice site shown in bold, ATG start of GS1 underlined.

GTTTGAATCCTCCTTAAAGTTTTTCTCTGGAGAAACTGTAGTAATTTTACTTTGTT

GTGTTCCCTTCATCTTTTGAATTAATGGCATTTGTTTTAATACTAATCTGCTTCTG

AAACTTGTAATGTATGTATATCAGTTTCTTATAATTTATCCAAGTAATATCTTCCAT

TCTCTATGCAATTGCCTGCATAAGCTCGACAAAAGAGTACATCAACCCCTCCTCC

TCTGGACTACTCTAGCTAAACTTGAATTTCCCCTTAAGATTATGAAATTGATATAT

CCTTAACAAACGACTCCTTCTGTTGGAAAATGTAGTACTTGTCTTTCTTCTTTTGG

GTATATATAGTTTATATACACCATACTATGTACAACATCCAAGTAGAGTGAAATG

GATACATGTACAAGACTTATTTGATTGATTGATGACTTGAGTTGCCTTAGGAGTA

ACAAATTCTTAGGTCAATAAATCGTTGATTTGAAATTAATCTCTCTGTCTTAGACA

GATAGGAATTATGACTTCCAATGGTCCAGAAAGCAAAGTTCGCACTGAGGGTAT

ACTTGGAATTGAGACTTGCACAGGTCCAGAAACCAAAGTTCCCATCGAGCTCTA

AAATCACATCTTTGGAATGAAATTCAATTAGAGATAAGTTGCTTCATAGCATAGG

TAAAATGGAAGATGTGAAGTAACCTGCAATAATCAGTGAAATGACATTAATACAC

TAAATACTTCATATGTAATTATCCTTTCCAGGTTAACAATACTCTATAAAGTAAGA

ATTATCAGAAATGGGCTCATCAAACTTTTGTACTATGTATTTCATATAAGGAAGTA

TAACTATACATAAGTGTATACACAACTTTATTCCTATTTTGTAAAGGTGGAGAGAC

TGTTTTCGATGGATCTAAAGCAATATGTCTATAAAATGCATTGATATAATAATTAT

CTGAGAAAATCCAGAATTGGCGTTGGATTATTTCAGCCAAATAGAAGTTTGTACC

ATACTTGTTGATTCCTTCTAAGTTAAGGTGAAGTATCATTCATAAACAGTTTTCCC

CAAAGTACTACTCACCAAGTTTCCCTTTGTAGAATTAACAGTTCAAATATATGGC

GCAGAAATTACTCTATGCCCAAAACCAAACGAGAAAGAAACAAAATACAGGGGT

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TGCAGACTTTATTTTCGTGTTAGGGTGTGTTTTTTCATGTAATTAATCAAAAAATA

TTATGACAAAAACATTTATACATATTTTTACTCAACACTCTGGGTATCAGGGTGG

GTTGTGTTCGACAATCAATATGGAAAGGAAGTATTTTCCTTATTTTTTTAGTTAAT

ATTTTCAGTTATACCAAACATACCTTGTGATATTATTTTTAAAAATGAAAAACTCGT

CAGAAAGAAAAAGCAAAAGCAACAAAAAAATTGCAAGTATTTTTTAAAAAAGAAA

AAAAAAACATATCTTGTTTGTCAGTATGGGAAGTTTGAGATAAGGACGAGTGAG

GGGTTAAAATTCAGTGGCCATTGATTTTGTAATGCCAAGAACCACAAAATCCAAT

GGTTACCATTCCTGTAAGATGAGGTTTGCTAACTCTTTTTGTCCGTTAGATAGGA

AGCCTTATCACTATATATACAAGGCGTCCTAATAACCTCTTAGTAACCAATTATTT

CAGCA**CC**ATG**T**CTCTGCTCTCAGATCTCGTTAACCTCAACCTCACCGATGCCAC

CGGGAAAATCATCGCCGAATACATATGGATCGGTGGATCTGGAATGGATATCAG

AAGCAAAGCCAGGACACTACCAGGACCAGTGACTGATCCATCAAAGCTTCCCAA

GTGGAACTACGACGGATCCAGCACCGGTCAGGCTGCTGGAGAAGACAGTGAAG

TCATTCTATACCCTCAGGCAATATTCAAGGATCCCTTCAGGAAAGGCAACAACAT

CCTGGTGATGTGTGATGCTTACACACCAGCTGGTGATCCTATTCCAACCAACAA

GAGGCACAACGCTGCTAAGATCTTCAGCCACCCCGACGTTGCCAAGGAGGAGC

CTTGGTATGGGATTGAGCAAGAATACACTTTGATGCAAAAGGATGTGAACTGGC

CAATTGGTTGGCCTGTTGGTGGCTACCCTGGCCCTCAGGGACCTTACTACTGTG

GTGTGGGAGCTGACAAAGCCATTGGTCGTGACATTGTGGATGCTCACTACAAG

GCCTGTCTTTACGCCGGTATTGGTATTTCTGGTATCAATGGAGAAGTCATGCCA

GGCCAGTGGGAGTTCCAAGTCGGCCCTGTTGAGGGTATTAGTTCTGGTGATCA

AGTCTGGGTTGCTCGATACCTTCTCGAGAGGATCACTGAGATCTCTGGTGTAAT

TGTCAGCTTCGACCCGAAACCAGTCCCGGGTGACTGGAATGGAGCTGGAGCTC

ACTGCAACTACAGCACTAAGACAATGAGAAACGATGGAGGATTAGAAGTGATCA

AGAAAGCGATAGGGAAGCTTCAGCTGAAACACAAAGAACACATTGCTGCTTACG

GTGAAGGAAACGAGCGTCGTCTCACTGGAAAGCACGAAACCGCAGACATCAAC

ACATTCTCTTGGGGAGTCGCGAACCGTGGAGCGTCAGTGAGAGTGGGACGTGA

CACAGAGAAGGAAGGTAAAGGGTACTTCGAAGACAGAAGGCCAGCTTCTAACAT

GGATCCTTACGTTGTCACCTCCATGATCGCTGAGACGACCATACTCGGTTGA

SEQ ID NO: 48: Putative Clementine orange GPT coding sequence
Derived from BioChain (Hayward, CA orange cDNA library, cat# C1634340;
Derived from clementine PCR primers:
5'-ggccacatgtccgttgctaagtgcttggagaagttta-3' (AflIII oligo) [SEQ ID NO: _]
5'-cgggcacgtgtcattttctcctcagcttctccttcatcct-3' (PmlI oligo) [SEQ ID NO: _]
ATG start site in bold, AflIII oligo binding site (start of putative mature coding sequence) is underlined; terminator sequence italicized.
ATGCTTAAGCCGTCCGCCTTCGGGTCTTCTTTTTCTTCCTCAGCTCTGCTTTCGT

TTTCGAAGCATTTGCATACAATAAGCATTACTGATTCTGTCAACACCAGAAGAAG

AGGAATCAGTACCGCTTGCCCTAGGTACCCTTCTCTCATGGCGAGCTTGTCCAC

CGTTTCCACCAATCAAAGCGACACCATCCAGAAGACCAATCTTCAGCCTCAACA

GGTTGCTAAGTGCTTGGAGAAGTTTAAAACTACAATCTTTACACAAATGAGTATG

CTTGCCATCAAACATGGAGCTATAAATCTTGGTCAAGGCTTTCCCAACTTTGATG

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GCCCAGATTTTGTTAAAGATGCAGCGATTCAAGCCATAAGGGATGGGAAGAATC

AATATGCTCGTGGACATGGGGTTCCAGAGTTCAACTCTGCCATTGCTTCCCGGT

TTAAGAAAGATTCTGGGCTCGAGGTTGACCCTGAAAAGGAAGTTACTGTTACCT

CTGGGTGCACCGAAGCCATTGCTGCAACCATCTTAGGTTTGATTAATCCTGGAG

ATGAGGTGATCCTTTTTGCACCTTTCTATGATTCCTATGAAGCTACTCTCTCCAT

GGCTGGTGCTAAAATTAAATGCATCACATTGCGCCCTCCAGAATTTGCCATCCC

CATTGAAGAGCTCAAGTCTACAATCTCAAAAAATACTCGTGCAATTCTTATGAAC

ACTCCACATAACCCCACTGGAAAGATGTTCACTAGGGAGGAACTTAATGTTATTG

CATCTCTTTGCATTGAGAATGATGTGTTGGTTTTTAGTGATGAGGTCTATGATAA

GTTGGCTTTTGAAATGGATCACATTTCCATAGCCTCTCTTCCTGGAATGTATGAG

CGTACTGTAACCATGAATTCCTTAGGGAAGACATTCTCTTTAACAGGGTGGAAG

ATCGGGTGGGCAATAGCTCCACCGCACCTTACATGGGGGGTGCGGCAGGCAC

ACTCTTTTCTCACGTTTGCCACATCCACTCCAATGCAGTGGGCAGCTACAGCAG

CCCTTAGAGCTCCGGAGACGTACTATGAGGAGCTAAAGAGAGATTACTCGGCAA

AGAAGGCAATTTTGGTGGAGGGATTGAATGCTGTTGGTTTCAAGGTATTCCCAT

CTAGTGGGACATACTTTGTGGTTGTAGATCACACCCCATTTGGGCACGAAACTG

ATATTGCATTTTGTGAATATCTGATCAAGGAAGTTGGGGTTGTGGCAATTCCGAC

CAGCGTATTTTACTTGAATCCAGAGGATGGAAAGAATTTGGTGAGATTTACCTTC

TGCAAAGATGAAGGAACTTTGAGGTCTGCAGTTGACAGGATGAAGGAGAAGCT

GAGGAGAAAA*TGA*

SEQ ID NO: 49: Putative Clementine orange GPT amino acid sequence; putative mature protein sequence begins at VAK shown in bold underline.
MLKPSAFGSSFSSSALLSFSKHLHTISITDSVNTRRRGISTACPRYPSLMASLSTVST

NQSDTIQKTNLQPQQ**VAK**CLEKFKTTIFTQMSMLAIKHGAINLGQGFPNFDGPDFVK

DAAIQAIRDGKNQYARGHGVPEFNSAIASRFKKDSGLEVDPEKEVTVTSGCTEAIAA

TILGLINPGDEVILFAPFYDSYEATLSMAGAKIKCITLRPPEFAIPIEELKSTISKNTRAIL

MNTPHNPTGKMFTREELNVIASLCIENDVLVFSDEVYDKLAFEMDHISIASLPGMYE

RTVTMNSLGKTFSLTGWKIGWAIAPPHLTWGVRQAHSFLTFATSTPMQWAATAALR

APETYYEELKRDYSAKKAILVEGLNAVGFKVFPSSGTYFVVVDHTPFGHETDIAFCE

YLIKEVGVVAIPTSVFYLNPEDGKNLVRFTFCKDEGTLRSAVDRMKEKLRRK

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 51

<210> SEQ ID NO 1
<211> LENGTH: 1323
<212> TYPE: DNA
<213> ORGANISM: Aradopsis thaliana

<400> SEQUENCE: 1

atgtacctgg acataaatgg tgtgatgatc aaacagttta gcttcaaagc ctctcttctc 60

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ccattctctt ctaatttccg acaaagctcc gccaaaatcc atcgtcctat cggagccacc    120
atgaccacag tttcgactca gaacgagtct actcaaaaac ccgtccaggt ggcgaagaga    180
ttagagaagt tcaagactac tattttcact caaatgagca tattggcagt taaacatgga    240
gcgatcaatt taggccaagg ctttcccaat ttcgacggtc ctgattttgt taaagaagct    300
gcgatccaag ctattaaaga tggtaaaaac cagtatgctc gtggatacgg cattcctcag    360
ctcaactctg ctatagctgc gcggtttcgt gaagatacgg gtcttgttgt tgatcctgag    420
aaagaagtta ctgttacatc tggttgcaca gaagccatag ctgcagctat gttgggttta    480
ataaaccctg gtgatgaagt cattctcttt gcaccgtttt atgattccta tgaagcaaca    540
ctctctatgg ctggtgctaa agtaaaagga atcactttac gtccaccgga cttctccatc    600
cctttggaag agcttaaagc tgcggtaact aacaagactc gagccatcct tatgaacact    660
ccgcacaacc cgaccgggaa gatgttcact agggaggagc ttgaaaccat tgcatctctc    720
tgcattgaaa acgatgtgct tgtgttctcg gatgaagtat acgataagct tgcgtttgaa    780
atggatcaca tttctatagc ttctcttccc ggtatgtatg aaagaactgt gaccatgaat    840
tccctgggaa agactttctc tttaaccgga tggaagatcg gctgggcgat tgcgccgcct    900
catctgactt ggggagttcg acaagcacac tcttacctca cattcgccac atcaacacca    960
gcacaatggg cagccgttgc agctctcaag gcaccagagt cttacttcaa agagctgaaa   1020
agagattaca atgtgaaaaa ggagactctg gttaagggtt tgaaggaagt cggatttaca   1080
gtgttcccat cgagcgggac ttactttgtg gttgctgatc acactccatt tggaatggag   1140
aacgatgttg ctttctgtga gtatcttatt gaagaagttg gggtcgttgc gatcccaacg   1200
agcgtctttt atctgaatcc agaagaaggg aagaatttgg ttaggtttgc gttctgtaaa   1260
gacgaagaga cgttgcgtgg tgcaattgag aggatgaagc agaagcttaa gagaaaagtc   1320
tga                                                                 1323
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<210> SEQ ID NO 2
<211> LENGTH: 440
<212> TYPE: PRT
<213> ORGANISM: Aradopsis thaliana

<400> SEQUENCE: 2

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Met Tyr Leu Asp Ile Asn Gly Val Met Ile Lys Gln Phe Ser Phe Lys
1               5                   10                  15

Ala Ser Leu Leu Pro Phe Ser Ser Asn Phe Arg Gln Ser Ser Ala Lys
            20                  25                  30

Ile His Arg Pro Ile Gly Ala Thr Met Thr Thr Val Ser Thr Gln Asn
        35                  40                  45

Glu Ser Thr Gln Lys Pro Val Gln Val Ala Lys Arg Leu Glu Lys Phe
    50                  55                  60

Lys Thr Thr Ile Phe Thr Gln Met Ser Ile Leu Ala Val Lys His Gly
65                  70                  75                  80

Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly Pro Asp Phe
                85                  90                  95

Val Lys Glu Ala Ala Ile Gln Ala Ile Lys Asp Gly Lys Asn Gln Tyr
            100                 105                 110

Ala Arg Gly Tyr Gly Ile Pro Gln Leu Asn Ser Ala Ile Ala Ala Arg
        115                 120                 125
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Phe Arg Glu Asp Thr Gly Leu Val Val Asp Pro Glu Lys Glu Val Thr
    130                 135                 140

Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Ala Met Leu Gly Leu
145                 150                 155                 160

Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe Tyr Asp Ser
                165                 170                 175

Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Lys Val Lys Gly Ile Thr
            180                 185                 190

Leu Arg Pro Pro Asp Phe Ser Ile Pro Leu Glu Glu Leu Lys Ala Ala
        195                 200                 205

Val Thr Asn Lys Thr Arg Ala Ile Leu Met Asn Thr Pro His Asn Pro
    210                 215                 220

Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Glu Thr Ile Ala Ser Leu
225                 230                 235                 240

Cys Ile Glu Asn Asp Val Leu Val Phe Ser Asp Glu Val Tyr Asp Lys
                245                 250                 255

Leu Ala Phe Glu Met Asp His Ile Ser Ile Ala Ser Leu Pro Gly Met
            260                 265                 270

Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr Phe Ser Leu
        275                 280                 285

Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro Pro His Leu Thr Trp
    290                 295                 300

Gly Val Arg Gln Ala His Ser Tyr Leu Thr Phe Ala Thr Ser Thr Pro
305                 310                 315                 320

Ala Gln Trp Ala Ala Val Ala Ala Leu Lys Ala Pro Glu Ser Tyr Phe
                325                 330                 335

Lys Glu Leu Lys Arg Asp Tyr Asn Val Lys Lys Glu Thr Leu Val Lys
            340                 345                 350

Gly Leu Lys Glu Val Gly Phe Thr Val Phe Pro Ser Ser Gly Thr Tyr
        355                 360                 365

Phe Val Val Ala Asp His Thr Pro Phe Gly Met Glu Asn Asp Val Ala
    370                 375                 380

Phe Cys Glu Tyr Leu Ile Glu Glu Val Gly Val Val Ala Ile Pro Thr
385                 390                 395                 400

Ser Val Phe Tyr Leu Asn Pro Glu Glu Gly Lys Asn Leu Val Arg Phe
                405                 410                 415

Ala Phe Cys Lys Asp Glu Glu Thr Leu Arg Gly Ala Ile Glu Arg Met
            420                 425                 430

Lys Gln Lys Leu Lys Arg Lys Val
        435                 440


<210> SEQ ID NO 3
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Medicago sativa

<400> SEQUENCE: 3

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aatacagatt ccttagattt tactaagttt gaaaccaaaa ccaaaacatg tctctccttt    120

cagatcttat caaccttgac ctctccgaaa ccaccgagaa aatcatcgcc gaatacatat    180

ggattggtgg atctggtttg gacttgagga gcaaagcaag gactctacca ggaccagtta    240
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ctgacccttc acagcttccc aagtggaact atgatggttc cagcacaggt caagctcctg    300

gagaagatag tgaagttatt atctacccac aagccatttt caaggaccca tttagaaggg    360

gtaacaatat cttggttatg tgtgatgcat acactccagc tggagagccc attcccacca    420

acaagagaca tgcagctgcc aagattttca gccatcctga tgttgttgct gaagtaccat    480

ggtatggtat tgagcaagaa tacaccttgt tgcagaaaga catcaattgg cctcttggtt    540

ggccagttgg tggttttcct ggacctcagg gaccatacta ttgtggagct ggtgctgaca    600

aggcatttgg ccgtgacatt gttgactcac attacaaagc ctgtctttat gccggcatca    660

acatcagtgg aatcaatggt gaagtgatgc ctggtcaatg ggaattccaa gttggtccct    720

cagttggtat ctctgctggt gatgagatat gggttgctcg ttacattttg gagaggatca    780

ctgaggttgc tggtgtggtg ctttcctttg acccaaaacc aattaagggt gattggaatg    840

gtgctggtgc tcacacaaat tacagcacca agtctatgag agaagatggt ggctatgaag    900

tcatcttgaa agcaattgag aagcttggga agaagcacaa ggagcacatt gctgcttatg    960

gagaaggcaa cgagcgtaga ttgacagggc gacatgagac agctgacatt aacaccttct   1020

tatggggtgt tgcaaaccgt ggtgcgtcga ttagagttgg aagggacaca gagaaagcag   1080

ggaaaggtta tttcgaggat aggaggccat catctaacat ggatccatat gttgttactt   1140

ccatgattgc agacaccacc attctctgga aaccataagc caccacacac acatgcattg   1200

aagtatttga aagtcattgt tgattccgca ttagaatttg gtcattgttt tttctaggat   1260

ttggatttgt gttattgtta tggttcacac tttgtttgtt tgaatttgag gccttgttat   1320

aggtttcata tttctttctc ttgttctaag taaatgtcag aataataatg taat         1374
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<210> SEQ ID NO 4
<211> LENGTH: 356
<212> TYPE: PRT
<213> ORGANISM: Medicago sativa

<400> SEQUENCE: 4

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Met Ser Leu Leu Ser Asp Leu Ile Asn Leu Asp Leu Ser Glu Thr Thr
1               5                   10                  15

Glu Lys Ile Ile Ala Glu Tyr Ile Trp Ile Gly Gly Ser Gly Leu Asp
            20                  25                  30

Leu Arg Ser Lys Ala Arg Thr Leu Pro Gly Pro Val Thr Asp Pro Ser
        35                  40                  45

Gln Leu Pro Lys Trp Asn Tyr Asp Gly Ser Ser Thr Gly Gln Ala Pro
    50                  55                  60

Gly Glu Asp Ser Glu Val Ile Ile Tyr Pro Gln Ala Ile Phe Lys Asp
65                  70                  75                  80

Pro Phe Arg Arg Gly Asn Asn Ile Leu Val Met Cys Asp Ala Tyr Thr
                85                  90                  95

Pro Ala Gly Glu Pro Ile Pro Thr Asn Lys Arg His Ala Ala Ala Lys
            100                 105                 110

Ile Phe Ser His Pro Asp Val Val Ala Glu Val Pro Trp Tyr Gly Ile
        115                 120                 125

Glu Gln Glu Tyr Thr Leu Leu Gln Lys Asp Ile Asn Trp Pro Leu Gly
    130                 135                 140

Trp Pro Val Gly Gly Phe Pro Gly Pro Gln Gly Pro Tyr Tyr Cys Gly
145                 150                 155                 160
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Ala Gly Ala Asp Lys Ala Phe Gly Arg Asp Ile Val Asp Ser His Tyr
                165                 170                 175

Lys Ala Cys Leu Tyr Ala Gly Ile Asn Ile Ser Gly Ile Asn Gly Glu
            180                 185                 190

Val Met Pro Gly Gln Trp Glu Phe Gln Val Gly Pro Ser Val Gly Ile
        195                 200                 205

Ser Ala Gly Asp Glu Ile Trp Val Ala Arg Tyr Ile Leu Glu Arg Ile
    210                 215                 220

Thr Glu Val Ala Gly Val Val Leu Ser Phe Asp Pro Lys Pro Ile Lys
225                 230                 235                 240

Gly Asp Trp Asn Gly Ala Gly Ala His Thr Asn Tyr Ser Thr Lys Ser
                245                 250                 255

Met Arg Glu Asp Gly Gly Tyr Glu Val Ile Leu Lys Ala Ile Glu Lys
            260                 265                 270

Leu Gly Lys Lys His Lys Glu His Ile Ala Ala Tyr Gly Glu Gly Asn
        275                 280                 285

Glu Arg Arg Leu Thr Gly Arg His Glu Thr Ala Asp Ile Asn Thr Phe
    290                 295                 300

Leu Trp Gly Val Ala Asn Arg Gly Ala Ser Ile Arg Val Gly Arg Asp
305                 310                 315                 320

Thr Glu Lys Ala Gly Lys Gly Tyr Phe Glu Asp Arg Arg Pro Ser Ser
                325                 330                 335

Asn Met Asp Pro Tyr Val Val Thr Ser Met Ile Ala Asp Thr Thr Ile
            340                 345                 350

Leu Trp Lys Pro
        355


<210> SEQ ID NO 5
<211> LENGTH: 1419
<212> TYPE: DNA
<213> ORGANISM: Medicago sativa

<400> SEQUENCE: 5

atcgatgaat tcgagctcgg tacccatttc cgttttcgtt ttcatttgat tcattgaatc     60

aaatcgaatc gaatctttag gattcaatac agattcctta gattttacta agtttgaaac    120

caaaaccaaa acatgtctct cctttcagat cttatcaacc ttgacctctc cgaaaccacc    180

gagaaaatca tcgccgaata catatggatt ggtggatctg gtttggactt gaggagcaaa    240

gcaaggactc taccaggacc agttactgac ccttcacagc ttcccaagtg gaactatgat    300

ggttccagca caggtcaagc tcctggagaa gatagtgaag ttattatcta cccacaagcc    360

attttcaagg acccatttag aaggggtaac aatatcttgg ttatgtgtga tgcatacact    420

ccagctggag agcccattcc caccaacaag agacatgcag ctgccaagat tttcagccat    480

cctgatgttg ttgctgaagt accatggtat ggtattgagc aagaatacac cttgttgcag    540

aaagacatca attggcctct tggttggcca gttggtggtt ttcctggacc tcagggacca    600

tactattgtg gagctggtgc tgacaaggca tttggccgtg acattgttga ctcacattac    660

aaagcctgtc tttatgccgg catcaacatc agtggaatca atggtgaagt gatgcctggt    720

caatgggaat tccaagttgg tccctcagtt ggtatctctg ctggtgatga gatatgggtt    780

gctcgttaca ttttggagag gatcactgag gttgctggtg tggtgctttc ctttgaccca    840

aaaccaatta agggtgattg gaatggtgct ggtgctcaca caaattacag caccaagtct    900
```

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atgagagaag atggtggcta tgaagtcatc ttgaaagcaa ttgagaagct tgggaagaag    960
cacaaggagc acattgctgc ttatggagaa ggcaacgagc gtagattgac agggcgacat   1020
gagacagctg acattaacac cttcttatgg ggtgttgcaa accgtggtgc gtcgattaga   1080
gttggaaggg acacagagaa agcagggaaa ggttatttcg aggataggag gccatcatct   1140
aacatggatc catatgttgt tacttccatg attgcagaca ccaccattct ctggaaacca   1200
taagccacca cacacacatg cattgaagta tttgaaagtc attgttgatt ccgcattaga   1260
atttggtcat tgtttttct aggatttgga tttgtgttat tgttatggtt cacactttgt   1320
ttgtttgaat ttgaggcctt gttataggtt tcatatttct ttctcttgtt ctaagtaaat   1380
gtcagaataa taatgtaatg gggatcctct agagtcgag                          1419
```

<210> SEQ ID NO 6
<211> LENGTH: 1302
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic plasmid vector sequence

<400> SEQUENCE: 6

```
aaaaaagaaa aaaaaaacat atcttgtttg tcagtatggg aagtttgaga taaggacgag     60
tgaggggtta aaattcagtg gccattgatt ttgtaatgcc aagaaccaca aaatccaatg    120
gttaccattc ctgtaagatg aggtttgcta actctttttg tccgttagat aggaagcctt    180
atcactatat atacaaggcg tcctaataac ctcttagtaa ccaattattt cagcaccatg    240
tctctgctct cagatctcgt taacctcaac ctcaccgatg ccaccgggaa aatcatcgcc    300
gaatacatat ggatcggtgg atctggaatg gatatcagaa gcaaagccag gacactacca    360
ggaccagtga ctgatccatc aaagcttccc aagtggaact acgacggatc cagcaccggt    420
caggctgctg gagaagacag tgaagtcatt ctataccctc aggcaatatt caaggatccc    480
ttcaggaaag gcaacaacat cctggtgatg tgtgatgctt acacaccagc tggtgatcct    540
attccaacca acaagaggca caacgctgct aagatcttca gccaccccga cgttgccaag    600
gaggagcctt ggtatgggat tgagcaagaa tacactttga tgcaaaagga tgtgaactgg    660
ccaattggtt ggcctgttgg tggctaccct ggccctcagg gaccttacta ctgtggtgtg    720
ggagctgaca aagccattgg tcgtgacatt gtggatgctc actacaaggc ctgtctttac    780
gccggtattg gtatttctgg tatcaatgga gaagtcatgc caggccagtg ggagttccaa    840
gtcggccctg ttgagggtat tagttctggt gatcaagtct gggttgctcg ataccttctc    900
gagaggatca ctgagatctc tggtgtaatt gtcagcttcg acccgaaacc agtcccgggt    960
gactggaatg gagctggagc tcactgcaac tacagcacta agacaatgag aaacgatgga   1020
ggattagaag tgatcaagaa agcgataggg aagcttcagc tgaaacacaa agaacacatt   1080
gctgcttacg gtgaaggaaa cgagcgtcgt ctcactggaa agcacgaaac cgcagacatc   1140
aacacattct cttggggagt cgcgaaccgt ggagcgtcag tgagagtggg acgtgacaca   1200
gagaaggaag gtaaagggta cttcgaagac agaaggccag cttctaacat ggatccttac   1260
gttgtcacct ccatgatcgc tgagacgacc atactcggtt ga                      1302
```

<210> SEQ ID NO 7
<211> LENGTH: 364

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<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)..(9)
<223> OTHER INFORMATION: amino terminal 9 amino acids vector encoded
sequence

<400> SEQUENCE: 7

Met Val Asp Leu Arg Asn Arg Arg Thr Ser Met Ser Leu Leu Ser Asp
1 5 10 15

Leu Val Asn Leu Asn Leu Thr Asp Ala Thr Gly Lys Ile Ile Ala Glu
20 25 30

Tyr Ile Trp Ile Gly Gly Ser Gly Met Asp Ile Arg Ser Lys Ala Arg
35 40 45

Thr Leu Pro Gly Pro Val Thr Asp Pro Ser Lys Leu Pro Lys Trp Asn
50 55 60

Tyr Asp Gly Ser Ser Thr Gly Gln Ala Ala Gly Glu Asp Ser Glu Val
65 70 75 80

Ile Leu Tyr Pro Gln Ala Ile Phe Lys Asp Pro Phe Arg Lys Gly Asn
85 90 95

Asn Ile Leu Val Met Cys Asp Ala Tyr Thr Pro Ala Gly Asp Pro Ile
100 105 110

Pro Thr Asn Lys Arg His Asn Ala Ala Lys Ile Phe Ser His Pro Asp
115 120 125

Val Ala Lys Glu Glu Pro Trp Tyr Gly Ile Glu Gln Glu Tyr Thr Leu
130 135 140

Met Gln Lys Asp Val Asn Trp Pro Ile Gly Trp Pro Val Gly Gly Tyr
145 150 155 160

Pro Gly Pro Gln Gly Pro Tyr Tyr Cys Gly Val Gly Ala Asp Lys Ala
165 170 175

Ile Gly Arg Asp Ile Val Asp Ala His Tyr Lys Ala Cys Leu Tyr Ala
180 185 190

Gly Ile Gly Ile Ser Gly Ile Asn Gly Glu Val Met Pro Gly Gln Trp
195 200 205

Glu Phe Gln Val Gly Pro Val Glu Gly Ile Ser Ser Gly Asp Gln Val
210 215 220

Trp Val Ala Arg Tyr Leu Leu Glu Arg Ile Thr Glu Ile Ser Gly Val
225 230 235 240

Ile Val Ser Phe Asp Pro Lys Pro Val Pro Gly Asp Trp Asn Gly Ala
245 250 255

Gly Ala His Cys Asn Tyr Ser Thr Lys Thr Met Arg Asn Asp Gly Gly
260 265 270

Leu Glu Val Ile Lys Lys Ala Ile Gly Lys Leu Gln Leu Lys His Lys
275 280 285

Glu His Ile Ala Ala Tyr Gly Glu Gly Asn Glu Arg Arg Leu Thr Gly
290 295 300

Lys His Glu Thr Ala Asp Ile Asn Thr Phe Ser Trp Gly Val Ala Asn
305 310 315 320

Arg Gly Ala Ser Val Arg Val Gly Arg Asp Thr Glu Lys Glu Gly Lys
325 330 335

Gly Tyr Phe Glu Asp Arg Arg Pro Ala Ser Asn Met Asp Pro Tyr Val
340 345 350

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Val Thr Ser Met Ile Ala Glu Thr Thr Ile Leu Gly
        355                 360


<210> SEQ ID NO 8
<211> LENGTH: 1817
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic plasmid vector sequence including
      vitis vinifera GPT coding sequence

<400> SEQUENCE: 8

aaaaaagaaa aaaaaaacat atcttgtttg tcagtatggg aagtttgaga taaggacgag     60

tgaggggtta aaattcagtg gccattgatt ttgtaatgcc aagaaccaca aaatccaatg    120

gttaccattc ctgtaagatg aggtttgcta actctttttg tccgttagat aggaagcctt    180

atcactatat atacaaggcg tcctaataac ctcttagtaa ccaattattt cagcaccatg    240

gtagatctga gggtaaattt ctagtttttc tccttcattt tcttggttag gaccctttc    300

tctttttatt tttttgagct ttgatctttc tttaaactga tctatttttt aattgattgg    360

ttatggtgta aatattacat agctttaact gataatctga ttactttatt tcgtgtgtct    420

atgatgatga tgatagttac agaaccgacg aactagtatg cagctctctc aatgtacctg    480

gacattccca gagttgctta aaagaccagc ctttttaagg aggagtattg atagtatttc    540

gagtagaagt aggtccagct ccaagtatcc atctttcatg gcgtccgcat caacggtctc    600

cgctccaaat acggaggctg agcagaccca taaccccct caacctctac aggttgcaaa    660

gcgcttggag aaattcaaaa caacaatctt tactcaaatg agcatgcttg ccatcaaaca    720

tggagcaata aaccttggcc aagggtttcc caactttgat ggtcctgagt ttgtcaaaga    780

agcagcaatt caagccatta aggatgggaa aaaccaatat gctcgtggat atggagttcc    840

tgatctcaac tctgctgttg ctgatagatt caagaaggat acaggactcg tggtggaccc    900

cgagaaggaa gttactgtta cttctggatg tacagaagca attgctgcta ctatgctagg    960

cttgataaat cctggtgatg aggtgatcct ctttgctcca ttttatgatt cctatgaagc   1020

cactctatcc atggctggtg cccaaataaa atccatcact ttacgtcctc cggattttgc   1080

tgtgcccatg gatgagctca agtctgcaat ctcaaagaat acccgtgcaa tccttataaa   1140

cactccccat aaccccacag gaaagatgtt cacaagggag gaactgaatg tgattgcatc   1200

cctctgcatt gagaatgatg tgttggtgtt tactgatgaa gtttacgaca agttggcttt   1260

cgaaatggat cacatttcca tggcttctct tcctgggatg tacgagagga ccgtgactat   1320

gaattcctta gggaaaactt tctccctgac tggatggaag attggttgga cagtagctcc   1380

cccacacctg acatggggag tgaggcaagc ccactcattc ctcacgtttg ctacctgcac   1440

cccaatgcaa tgggcagctg caacagccct ccgggcccca gactcttact atgaagagct   1500

aaagagagat tacagtgcaa agaaggcaat cctggtggag ggattgaagg ctgtcggttt   1560

cagggtatac ccatcaagtg ggacctattt tgtggtggtg gatcacaccc catttgggtt   1620

gaaagacgat attgcgtttt gtgagtatct gatcaaggaa gttggggtgg tagcaattcc   1680

gacaagcgtt ttctacttac acccagaaga tggaaagaac cttgtgaggt ttaccttctg   1740

taaagacgag ggaactctga gagctgcagt tgaaaggatg aaggagaaac tgaagcctaa   1800

acaatagggg cacgtga                                                  1817
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<210> SEQ ID NO 9
<211> LENGTH: 459
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 9

Met Val Asp Leu Arg Asn Arg Arg Thr Ser Met Gln Leu Ser Gln Cys
1 5 10 15

Thr Trp Thr Phe Pro Glu Leu Leu Lys Arg Pro Ala Phe Leu Arg Arg
20 25 30

Ser Ile Asp Ser Ile Ser Ser Arg Ser Arg Ser Ser Ser Lys Tyr Pro
35 40 45

Ser Phe Met Ala Ser Ala Ser Thr Val Ser Ala Pro Asn Thr Glu Ala
50 55 60

Glu Gln Thr His Asn Pro Pro Gln Pro Leu Gln Val Ala Lys Arg Leu
65 70 75 80

Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Met Leu Ala Ile
85 90 95

Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly
100 105 110

Pro Glu Phe Val Lys Glu Ala Ala Ile Gln Ala Ile Lys Asp Gly Lys
115 120 125

Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Asp Leu Asn Ser Ala Val
130 135 140

Ala Asp Arg Phe Lys Lys Asp Thr Gly Leu Val Val Asp Pro Glu Lys
145 150 155 160

Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Thr Met
165 170 175

Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe
180 185 190

Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Gln Ile Lys
195 200 205

Ser Ile Thr Leu Arg Pro Pro Asp Phe Ala Val Pro Met Asp Glu Leu
210 215 220

Lys Ser Ala Ile Ser Lys Asn Thr Arg Ala Ile Leu Ile Asn Thr Pro
225 230 235 240

His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Asn Val Ile
245 250 255

Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val Phe Thr Asp Glu Val
260 265 270

Tyr Asp Lys Leu Ala Phe Glu Met Asp His Ile Ser Met Ala Ser Leu
275 280 285

Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr
290 295 300

Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Thr Val Ala Pro Pro His
305 310 315 320

Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe Leu Thr Phe Ala Thr
325 330 335

Cys Thr Pro Met Gln Trp Ala Ala Ala Thr Ala Leu Arg Ala Pro Asp
340 345 350

Ser Tyr Tyr Glu Glu Leu Lys Arg Asp Tyr Ser Ala Lys Lys Ala Ile
355 360 365

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Leu Val Glu Gly Leu Lys Ala Val Gly Phe Arg Val Tyr Pro Ser Ser
    370                 375                 380

Gly Thr Tyr Phe Val Val Val Asp His Thr Pro Phe Gly Leu Lys Asp
385                 390                 395                 400

Asp Ile Ala Phe Cys Glu Tyr Leu Ile Lys Glu Val Gly Val Val Ala
                405                 410                 415

Ile Pro Thr Ser Val Phe Tyr Leu His Pro Glu Asp Gly Lys Asn Leu
            420                 425                 430

Val Arg Phe Thr Phe Cys Lys Asp Glu Gly Thr Leu Arg Ala Ala Val
        435                 440                 445

Glu Arg Met Lys Glu Lys Leu Lys Pro Lys Gln
    450                 455


<210> SEQ ID NO 10
<211> LENGTH: 1446
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA encoding Oryza sativa GPT
      protein, codons optimized for expression in E. coli

<400> SEQUENCE: 10

atgtggatga acctggcagg ctttctggca accccggcaa ccgcaaccgc aacccgtcat     60

gaaatgccgc tgaacccgag cagcagcgcg agctttctgc tgagcagcct gcgtcgtagc    120

ctggtggcga gcctgcgtaa agcgagcccg gcagcagcag cagcactgag cccgatggca    180

agcgcaagca ccgtggcagc agaaaacggt gcagcaaaag cagcagcaga aaaacagcag    240

cagcagccgg tgcaggtggc gaaacgtctg gaaaaattta aaaccaccat ttttacccag    300

atgagcatgc tggcgattaa acatggcgcg attaacctgg gccagggctt tccgaacttt    360

gatggcccgg attttgtgaa agaagcggcg attcaggcga ttaacgcggg caaaaaccag    420

tatgcgcgtg gctatggcgt gccggaactg aacagcgcga ttgcggaacg ttttctgaaa    480

gatagcggcc tgcaggtgga tccggaaaaa gaagtgaccg tgaccagcgg ctgcaccgaa    540

gcgattgcgg cgaccattct gggcctgatt aacccgggcg atgaagtgat tctgtttgcg    600

ccgttttatg atagctatga agcgaccctg agcatggcgg gcgcgaacgt gaaagcgatt    660

accctgcgtc cgccggattt tagcgtgccg ctggaagaac tgaaagcggc cgtgagcaaa    720

aacacccgtg cgattatgat taacaccccg cataacccga ccggcaaaat gtttacccgt    780

gaagaactgg aatttattgc gaccctgtgc aaagaaaacg atgtgctgct gtttgcggat    840

gaagtgtatg ataaactggc gtttgaagcg gatcatatta gcatggcgag cattccgggc    900

atgtatgaac gtaccgtgac catgaacagc ctgggcaaaa cctttagcct gaccggctgg    960

aaaattggct gggcgattgc gccgccgcat ctgacctggg gcgtgcgtca ggcacatagc   1020

tttctgacct ttgcaacctg caccccgatg caggcagccg ccgcagcagc actgcgtgca   1080

ccggatagct attatgaaga actgcgtcgt gattatggcg cgaaaaaagc gctgctggtg   1140

aacggcctga aagatgcggg ctttattgtg tatccgagca gcggcaccta ttttgtgatg   1200

gtggatcata ccccgtttgg ctttgataac gatattgaat tttgcgaata tctgattcgt   1260

gaagtgggcg tggtggcgat tccgccgagc gtgttttatc tgaacccgga agatggcaaa   1320

aacctggtgc gttttacctt ttgcaaagat gatgaaaccc tgcgtgcggc ggtggaacgt   1380
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atgaaaacca aactgcgtaa aaaaaagctt gcggccgcac tcgagcacca ccaccaccac   1440

cactga                                                              1446


<210> SEQ ID NO 11
<211> LENGTH: 481
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oryza sativa GPT protein sequence with amino-
      and carboxyl-terminal vector sequences

<400> SEQUENCE: 11

Met Trp Met Asn Leu Ala Gly Phe Leu Ala Thr Pro Ala Thr Ala Thr
1               5                   10                  15

Ala Thr Arg His Glu Met Pro Leu Asn Pro Ser Ser Ser Ala Ser Phe
            20                  25                  30

Leu Leu Ser Ser Leu Arg Arg Ser Leu Val Ala Ser Leu Arg Lys Ala
        35                  40                  45

Ser Pro Ala Ala Ala Ala Ala Leu Ser Pro Met Ala Ser Ala Ser Thr
    50                  55                  60

Val Ala Ala Glu Asn Gly Ala Ala Lys Ala Ala Ala Glu Lys Gln Gln
65                  70                  75                  80

Gln Gln Pro Val Gln Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr
                85                  90                  95

Ile Phe Thr Gln Met Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn
            100                 105                 110

Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu
        115                 120                 125

Ala Ala Ile Gln Ala Ile Asn Ala Gly Lys Asn Gln Tyr Ala Arg Gly
    130                 135                 140

Tyr Gly Val Pro Glu Leu Asn Ser Ala Ile Ala Glu Arg Phe Leu Lys
145                 150                 155                 160

Asp Ser Gly Leu Gln Val Asp Pro Glu Lys Glu Val Thr Val Thr Ser
                165                 170                 175

Gly Cys Thr Glu Ala Ile Ala Ala Thr Ile Leu Gly Leu Ile Asn Pro
            180                 185                 190

Gly Asp Glu Val Ile Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala
        195                 200                 205

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

Pro Asp Phe Ser Val Pro Leu Glu Glu Leu Lys Ala Ala Val Ser Lys
225                 230                 235                 240

Asn Thr Arg Ala Ile Met Ile Asn Thr Pro His Asn Pro Thr Gly Lys
                245                 250                 255

Met Phe Thr Arg Glu Glu Leu Glu Phe Ile Ala Thr Leu Cys Lys Glu
            260                 265                 270

Asn Asp Val Leu Leu Phe Ala Asp Glu Val Tyr Asp Lys Leu Ala Phe
        275                 280                 285

Glu Ala Asp His Ile Ser Met Ala Ser Ile Pro Gly Met Tyr Glu Arg
    290                 295                 300

Thr Val Thr Met Asn Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp
305                 310                 315                 320

Lys Ile Gly Trp Ala Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg
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                325                 330                 335

Gln Ala His Ser Phe Leu Thr Phe Ala Thr Cys Thr Pro Met Gln Ala
            340                 345                 350

Ala Ala Ala Ala Ala Leu Arg Ala Pro Asp Ser Tyr Tyr Glu Glu Leu
        355                 360                 365

Arg Arg Asp Tyr Gly Ala Lys Lys Ala Leu Leu Val Asn Gly Leu Lys
    370                 375                 380

Asp Ala Gly Phe Ile Val Tyr Pro Ser Ser Gly Thr Tyr Phe Val Met
385                 390                 395                 400

Val Asp His Thr Pro Phe Gly Phe Asp Asn Asp Ile Glu Phe Cys Glu
                405                 410                 415

Tyr Leu Ile Arg Glu Val Gly Val Val Ala Ile Pro Pro Ser Val Phe
            420                 425                 430

Tyr Leu Asn Pro Glu Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys
        435                 440                 445

Lys Asp Asp Glu Thr Leu Arg Ala Ala Val Glu Arg Met Lys Thr Lys
    450                 455                 460

Leu Arg Lys Lys Lys Leu Ala Ala Ala Leu Glu His His His His His
465                 470                 475                 480

His


<210> SEQ ID NO 12
<211> LENGTH: 1251
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA encoding Glycine max GPT protein,
      codons optimized for expression in E. coli

<400> SEQUENCE: 12

atgcatcatc accatcacca tggtaagcct atccctaacc ctctcctcgg tctcgattct     60

acggaaaacc tgtattttca gggaattgat cccttcaccg cgaaacgtct ggaaaaattt    120

cagaccacca tttttaccca gatgagcctg ctggcgatta aacatggcgc gattaacctg    180

ggccagggct ttccgaactt tgatggcccg gaatttgtga aagaagcggc gattcaggcg    240

attcgtgatg gcaaaaacca gtatgcgcgt ggctatggcg tgccggatct gaacattgcg    300

attgcggaac gttttaaaaa agataccggc ctggtggtgg atccggaaaa agaaattacc    360

gtgaccagcg gctgcaccga agcgattgcg gcgaccatga ttggcctgat taacccgggc    420

gatgaagtga ttatgtttgc gccgttttat gatagctatg aagcgaccct gagcatggcg    480

ggcgcgaaag tgaaaggcat taccctgcgt ccgccggatt ttgcggtgcc gctggaagaa    540

ctgaaaagca ccattagcaa aaacacccgt gcgattctga ttaacacccc gcataacccg    600

accggcaaaa tgtttacccg tgaagaactg aactgcattg cgagcctgtg cattgaaaac    660

gatgtgctgg tgtttaccga tgaagtgtat gataaactgg cgtttgatat ggaacatatt    720

agcatggcga gcctgccggg catgtttgaa cgtaccgtga ccctgaacag cctgggcaaa    780

acctttagcc tgaccggctg gaaaattggc tgggcgattg cgccgccgca tctgagctgg    840

ggcgtgcgtc aggcgcatgc gtttctgacc tttgcaaccg cacatccgtt tcagtgcgca    900

gcagcagcag cactgcgtgc accggatagc tattatgtgg aactgaaacg tgattatatg    960

gcgaaacgtg cgattctgat tgaaggcctg aaagcggtgg gctttaaagt gtttccgagc   1020
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agcggcacct attttgtggt ggtggatcat accccgtttg gcctggaaaa cgatgtggcg   1080

ttttgcgaat atctggtgaa agaagtgggc gtggtggcga ttccgaccag cgtgttttat   1140

ctgaacccgg aagaaggcaa aaacctggtg cgttttacct tttgcaaaga tgaagaaacc   1200

attcgtagcg cggtggaacg tatgaaagcg aaactgcgta aagtcgacta a            1251


<210> SEQ ID NO 13
<211> LENGTH: 416
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Glycine max GPT amino acid sequence and
      amino-terminal vector sequence

<400> SEQUENCE: 13

Met His His His His His His Gly Lys Pro Ile Pro Asn Pro Leu Leu
1               5                   10                  15

Gly Leu Asp Ser Thr Glu Asn Leu Tyr Phe Gln Gly Ile Asp Pro Phe
            20                  25                  30

Thr Ala Lys Arg Leu Glu Lys Phe Gln Thr Thr Ile Phe Thr Gln Met
        35                  40                  45

Ser Leu Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
    50                  55                  60

Pro Asn Phe Asp Gly Pro Glu Phe Val Lys Glu Ala Ala Ile Gln Ala
65                  70                  75                  80

Ile Arg Asp Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Asp
                85                  90                  95

Leu Asn Ile Ala Ile Ala Glu Arg Phe Lys Lys Asp Thr Gly Leu Val
            100                 105                 110

Val Asp Pro Glu Lys Glu Ile Thr Val Thr Ser Gly Cys Thr Glu Ala
        115                 120                 125

Ile Ala Ala Thr Met Ile Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
    130                 135                 140

Met Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
145                 150                 155                 160

Gly Ala Lys Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe Ala Val
                165                 170                 175

Pro Leu Glu Glu Leu Lys Ser Thr Ile Ser Lys Asn Thr Arg Ala Ile
            180                 185                 190

Leu Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
        195                 200                 205

Glu Leu Asn Cys Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val
    210                 215                 220

Phe Thr Asp Glu Val Tyr Asp Lys Leu Ala Phe Asp Met Glu His Ile
225                 230                 235                 240

Ser Met Ala Ser Leu Pro Gly Met Phe Glu Arg Thr Val Thr Leu Asn
                245                 250                 255

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
            260                 265                 270

Ile Ala Pro Pro His Leu Ser Trp Gly Val Arg Gln Ala His Ala Phe
        275                 280                 285

Leu Thr Phe Ala Thr Ala His Pro Phe Gln Cys Ala Ala Ala Ala Ala
    290                 295                 300
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Leu Arg Ala Pro Asp Ser Tyr Tyr Val Glu Leu Lys Arg Asp Tyr Met
305                 310                 315                 320

Ala Lys Arg Ala Ile Leu Ile Glu Gly Leu Lys Ala Val Gly Phe Lys
                325                 330                 335

Val Phe Pro Ser Ser Gly Thr Tyr Phe Val Val Val Asp His Thr Pro
            340                 345                 350

Phe Gly Leu Glu Asn Asp Val Ala Phe Cys Glu Tyr Leu Val Lys Glu
        355                 360                 365

Val Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu
    370                 375                 380

Glu Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Glu Glu Thr
385                 390                 395                 400

Ile Arg Ser Ala Val Glu Arg Met Lys Ala Lys Leu Arg Lys Val Asp
                405                 410                 415


<210> SEQ ID NO 14
<211> LENGTH: 1278
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 14

atggtagatc tgaggaaccg acgaactagt atggcatccg cccccgcctc cgcctccgcg     60

gccctctcca ccgccgcccc cgccgacaac ggggccgcca agcccacgga gcagcggccg    120

gtacaggtgg ctaagcgatt ggagaagttc aaaacaacaa ttttcacaca gatgagcatg    180

ctcgcagtga agcatggagc aataaacctt ggacaggggt ttcccaattt tgatggccct    240

gactttgtca aagatgctgc tattgaggct atcaaagctg gaaagaatca gtatgcaaga    300

ggatatggtg tgcctgaatt gaactcagct gttgctgaga gatttctcaa ggacagtgga    360

ttgcacatcg atcctgataa ggaagttact gttacatctg ggtgcacaga agcaatagct    420

gcaacgatat tgggtctgat caaccctggg gatgaagtca tactgtttgc tccattctat    480

gattcttatg aggctacact gtccatggct ggtgcgaatg tcaaagccat tacactccgc    540

cctccggact ttgcagtccc tcttgaagag ctaaaggctg cagtctcgaa gaataccaga    600

gcaataatga ttaatacacc tcacaaccct accgggaaaa tgttcacaag ggaggaactt    660

gagttcattg ctgatctctg caaggaaaat gacgtgttgc tctttgccga tgaggtctac    720

gacaagctgg cgtttgaggc ggatcacata tcaatggctt ctattcctgg catgtatgag    780

aggaccgtca ctatgaactc cctggggaag acgttctcct tgaccggatg gaagatcggc    840

tgggcgatag caccaccgca cctgacatgg ggcgtaaggc aggcacactc cttcctcaca    900

ttcgccacct ccacgccgat gcaatcagca gcggcggcgg ccctgagagc accggacagc    960

tactttgagg agctgaagag ggactacggc gcaaagaaag cgctgctggt ggacgggctc   1020

aaggcggcgg gcttcatcgt ctacccttcg agcggaacct acttcatcat ggtcgaccac   1080

accccgttcg ggttcgacaa cgacgtcgag ttctgcgagt acttgatccg cgaggtcggc   1140

gtcgtggcca tcccgccaag cgtgttctac ctgaacccgg aggacgggaa gaacctggtg   1200

aggttcacct tctgcaagga cgacgacacg ctaagggcgg cggtggacag gatgaaggcc   1260

aagctcagga agaaatga                                                 1278


<210> SEQ ID NO 15
<211> LENGTH: 425
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-continued

<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 15

Met Val Asp Leu Arg Asn Arg Arg Thr Ser Met Ala Ser Ala Pro Ala
1 5 10 15

Ser Ala Ser Ala Ala Leu Ser Thr Ala Ala Pro Ala Asp Asn Gly Ala
20 25 30

Ala Lys Pro Thr Glu Gln Arg Pro Val Gln Val Ala Lys Arg Leu Glu
35 40 45

Lys Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Met Leu Ala Val Lys
50 55 60

His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly Pro
65 70 75 80

Asp Phe Val Lys Asp Ala Ala Ile Glu Ala Ile Lys Ala Gly Lys Asn
85 90 95

Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu Leu Asn Ser Ala Val Ala
100 105 110

Glu Arg Phe Leu Lys Asp Ser Gly Leu His Ile Asp Pro Asp Lys Glu
115 120 125

Val Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Thr Ile Leu
130 135 140

Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe Tyr
145 150 155 160

Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Asn Val Lys Ala
165 170 175

Ile Thr Leu Arg Pro Pro Asp Phe Ala Val Pro Leu Glu Glu Leu Lys
180 185 190

Ala Ala Val Ser Lys Asn Thr Arg Ala Ile Met Ile Asn Thr Pro His
195 200 205

Asn Pro Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Glu Phe Ile Ala
210 215 220

Asp Leu Cys Lys Glu Asn Asp Val Leu Leu Phe Ala Asp Glu Val Tyr
225 230 235 240

Asp Lys Leu Ala Phe Glu Ala Asp His Ile Ser Met Ala Ser Ile Pro
245 250 255

Gly Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr Phe
260 265 270

Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro Pro His Leu
275 280 285

Thr Trp Gly Val Arg Gln Ala His Ser Phe Leu Thr Phe Ala Thr Ser
290 295 300

Thr Pro Met Gln Ser Ala Ala Ala Ala Ala Leu Arg Ala Pro Asp Ser
305 310 315 320

Tyr Phe Glu Glu Leu Lys Arg Asp Tyr Gly Ala Lys Lys Ala Leu Leu
325 330 335

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

Thr Tyr Phe Ile Met Val Asp His Thr Pro Phe Gly Phe Asp Asn Asp
355 360 365

Val Glu Phe Cys Glu Tyr Leu Ile Arg Glu Val Gly Val Val Ala Ile
370 375 380

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Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu Asp Gly Lys Asn Leu Val
385                 390                 395                 400

Arg Phe Thr Phe Cys Lys Asp Asp Asp Thr Leu Arg Ala Ala Val Asp
                405                 410                 415

Arg Met Lys Ala Lys Leu Arg Lys Lys
            420                 425


<210> SEQ ID NO 16
<211> LENGTH: 1200
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA encoding Danio rerio GPT protein,
      codons optimized for expression in E. coli, including 5' and 3'
      vector sequences

<400> SEQUENCE: 16

atgtccgtgg cgaaacgtct ggaaaaattt aaaaccacca tttttaccca gatgagcatg     60

ctggcgatta aacatggcgc gattaacctg ggccagggct ttccgaactt tgatggcccg    120

gattttgtga aagaagcggc gattcaggcg attcgtgatg gcaacaacca gtatgcgcgt    180

ggctatggcg tgccggatct gaacattgcg attagcgaac gttataaaaa agataccggc    240

ctggcggtgg atccggaaaa agaaattacc gtgaccagcg gctgcaccga agcgattgcg    300

gcgaccgtgc tgggcctgat taacccgggc gatgaagtga ttgtgtttgc gccgttttat    360

gatagctatg aagcgaccct gagcatggcg ggcgcgaaag tgaaaggcat taccctgcgt    420

ccgccggatt ttgcgctgcc gattgaagaa ctgaaaagca ccattagcaa aaacacccgt    480

gcgattctgc tgaacacccc gcataacccg accggcaaaa tgtttacccc ggaagaactg    540

aacaccattg cgagcctgtg cattgaaaac gatgtgctgg tgtttagcga tgaagtgtat    600

gataaactgg cgtttgatat ggaacatatt agcattgcga gcctgccggg catgtttgaa    660

cgtaccgtga ccatgaacag cctgggcaaa acctttagcc tgaccggctg gaaaattggc    720

tgggcgattg cgccgccgca tctgacctgg ggcgtgcgtc aggcgcatgc gtttctgacc    780

tttgcaacca gcaacccgat gcagtgggca gcagcagtgg cactgcgtgc accggatagc    840

tattataccg aactgaaacg tgattatatg gcgaaacgta gcattctggt ggaaggcctg    900

aaagcggtgg gctttaaagt gtttccgagc agcggcacct attttgtggt ggtggatcat    960

accccgtttg gccatgaaaa cgatattgcg ttttgcgaat atctggtgaa agaagtgggc   1020

gtggtggcga ttccgaccag cgtgttttat ctgaacccgg aagaaggcaa aaacctggtg   1080

cgttttacct tttgcaaaga tgaaggcacc ctgcgtgcgg cggtggatcg tatgaaagaa   1140

aaactgcgta aagtcgacaa gcttgcggcc gcactcgagc accaccacca ccaccactga   1200


<210> SEQ ID NO 17
<211> LENGTH: 399
<212> TYPE: PRT
<213> ORGANISM: Danio rerio
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(399)
<223> OTHER INFORMATION: Amino- and carboxy-terminal amino acids shown

<400> SEQUENCE: 17

Met Ser Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr
1               5                   10                  15
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Gln Met Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln
            20                  25                  30

Gly Phe Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile
        35                  40                  45

Gln Ala Ile Arg Asp Gly Asn Asn Gln Tyr Ala Arg Gly Tyr Gly Val
    50                  55                  60

Pro Asp Leu Asn Ile Ala Ile Ser Glu Arg Tyr Lys Lys Asp Thr Gly
65                  70                  75                  80

Leu Ala Val Asp Pro Glu Lys Glu Ile Thr Val Thr Ser Gly Cys Thr
                85                  90                  95

Glu Ala Ile Ala Ala Thr Val Leu Gly Leu Ile Asn Pro Gly Asp Glu
            100                 105                 110

Val Ile Val Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser
        115                 120                 125

Met Ala Gly Ala Lys Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe
    130                 135                 140

Ala Leu Pro Ile Glu Glu Leu Lys Ser Thr Ile Ser Lys Asn Thr Arg
145                 150                 155                 160

Ala Ile Leu Leu Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr
                165                 170                 175

Pro Glu Glu Leu Asn Thr Ile Ala Ser Leu Cys Ile Glu Asn Asp Val
            180                 185                 190

Leu Val Phe Ser Asp Glu Val Tyr Asp Lys Leu Ala Phe Asp Met Glu
        195                 200                 205

His Ile Ser Ile Ala Ser Leu Pro Gly Met Phe Glu Arg Thr Val Thr
    210                 215                 220

Met Asn Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly
225                 230                 235                 240

Trp Ala Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His
                245                 250                 255

Ala Phe Leu Thr Phe Ala Thr Ser Asn Pro Met Gln Trp Ala Ala Ala
            260                 265                 270

Val Ala Leu Arg Ala Pro Asp Ser Tyr Tyr Thr Glu Leu Lys Arg Asp
        275                 280                 285

Tyr Met Ala Lys Arg Ser Ile Leu Val Glu Gly Leu Lys Ala Val Gly
    290                 295                 300

Phe Lys Val Phe Pro Ser Ser Gly Thr Tyr Phe Val Val Val Asp His
305                 310                 315                 320

Thr Pro Phe Gly His Glu Asn Asp Ile Ala Phe Cys Glu Tyr Leu Val
                325                 330                 335

Lys Glu Val Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn
            340                 345                 350

Pro Glu Glu Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Glu
        355                 360                 365

Gly Thr Leu Arg Ala Ala Val Asp Arg Met Lys Glu Lys Leu Arg Lys
    370                 375                 380

Val Asp Lys Leu Ala Ala Ala Leu Glu His His His His His His
385                 390                 395
```

<210> SEQ ID NO 18
<211> LENGTH: 1236

-continued

<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 18

atggccaaaa tccatcgtcc tatcggagcc accatgacca cagtttcgac tcagaacgag 60

tctactcaaa aacccgtcca ggtggcgaag agattagaga agttcaagac tactattttc 120

actcaaatga gcatattggc agttaaacat ggagcgatca atttaggcca aggctttccc 180

aatttcgacg gtcctgattt tgttaaagaa gctgcgatcc aagctattaa agatggtaaa 240

aaccagtatg ctcgtggata cggcattcct cagctcaact ctgctatagc tgcgcggttt 300

cgtgaagata cgggtcttgt tgttgatcct gagaaagaag ttactgttac atctggttgc 360

acagaagcca tagctgcagc tatgttgggt ttaataaacc ctggtgatga agtcattctc 420

tttgcaccgt tttatgattc ctatgaagca acactctcta tggctggtgc taaagtaaaa 480

ggaatcactt tacgtccacc ggacttctcc atccctttgg aagagcttaa agctgcggta 540

actaacaaga ctcgagccat ccttatgaac actccgcaca acccgaccgg gaagatgttc 600

actagggagg agcttgaaac cattgcatct ctctgcattg aaaacgatgt gcttgtgttc 660

tcggatgaag tatacgataa gcttgcgttt gaaatggatc acatttctat agcttctctt 720

cccggtatgt atgaaagaac tgtgaccatg aattccctgg gaaagacttt ctctttaacc 780

ggatggaaga tcggctgggc gattgcgccg cctcatctga cttggggagt tcgacaagca 840

cactcttacc tcacattcgc cacatcaaca ccagcacaat gggcagccgt tgcagctctc 900

aaggcaccag agtcttactt caaagagctg aaaagagatt acaatgtgaa aaaggagact 960

ctggttaagg gtttgaagga agtcggattt acagtgttcc catcgagcgg gacttacttt 1020

gtggttgctg atcacactcc atttggaatg gagaacgatg ttgctttctg tgagtatctt 1080

attgaagaag ttggggtcgt tgcgatccca acgagcgtct tttatctgaa tccagaagaa 1140

gggaagaatt tggttaggtt tgcgttctgt aaagacgaag agacgttgcg tggtgcaatt 1200

gagaggatga agcagaagct taagagaaaa gtctga 1236

<210> SEQ ID NO 19
<211> LENGTH: 411
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

Met Ala Lys Ile His Arg Pro Ile Gly Ala Thr Met Thr Thr Val Ser
1 5 10 15

Thr Gln Asn Glu Ser Thr Gln Lys Pro Val Gln Val Ala Lys Arg Leu
20 25 30

Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Ile Leu Ala Val
35 40 45

Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly
50 55 60

Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala Ile Lys Asp Gly Lys
65 70 75 80

Asn Gln Tyr Ala Arg Gly Tyr Gly Ile Pro Gln Leu Asn Ser Ala Ile
85 90 95

Ala Ala Arg Phe Arg Glu Asp Thr Gly Leu Val Val Asp Pro Glu Lys
100 105 110

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Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Ala Met
        115                 120                 125

Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe
    130                 135                 140

Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Lys Val Lys
145                 150                 155                 160

Gly Ile Thr Leu Arg Pro Pro Asp Phe Ser Ile Pro Leu Glu Glu Leu
                165                 170                 175

Lys Ala Ala Val Thr Asn Lys Thr Arg Ala Ile Leu Met Asn Thr Pro
            180                 185                 190

His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Glu Thr Ile
        195                 200                 205

Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val Phe Ser Asp Glu Val
    210                 215                 220

Tyr Asp Lys Leu Ala Phe Glu Met Asp His Ile Ser Ile Ala Ser Leu
225                 230                 235                 240

Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr
                245                 250                 255

Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro Pro His
            260                 265                 270

Leu Thr Trp Gly Val Arg Gln Ala His Ser Tyr Leu Thr Phe Ala Thr
        275                 280                 285

Ser Thr Pro Ala Gln Trp Ala Ala Val Ala Ala Leu Lys Ala Pro Glu
    290                 295                 300

Ser Tyr Phe Lys Glu Leu Lys Arg Asp Tyr Asn Val Lys Lys Glu Thr
305                 310                 315                 320

Leu Val Lys Gly Leu Lys Glu Val Gly Phe Thr Val Phe Pro Ser Ser
                325                 330                 335

Gly Thr Tyr Phe Val Val Ala Asp His Thr Pro Phe Gly Met Glu Asn
            340                 345                 350

Asp Val Ala Phe Cys Glu Tyr Leu Ile Glu Glu Val Gly Val Val Ala
        355                 360                 365

Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu Glu Gly Lys Asn Leu
    370                 375                 380

Val Arg Phe Ala Phe Cys Lys Asp Glu Glu Thr Leu Arg Gly Ala Ile
385                 390                 395                 400

Glu Arg Met Lys Gln Lys Leu Lys Arg Lys Val
                405                 410


<210> SEQ ID NO 20
<211> LENGTH: 1194
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 20

atggcgactc agaacgagtc tactcaaaaa cccgtccagg tggcgaagag attagagaag     60

ttcaagacta ctattttcac tcaaatgagc atattggcag ttaaacatgg agcgatcaat    120

ttaggccaag gctttcccaa tttcgacggt cctgattttg ttaaagaagc tgcgatccaa    180

gctattaaag atggtaaaaa ccagtatgct cgtggatacg gcattcctca gctcaactct    240

gctatagctg cgcggtttcg tgaagatacg ggtcttgttg ttgatcctga gaaagaagtt    300

actgttacat ctggttgcac agaagccata gctgcagcta tgttgggttt aataaaccct    360
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ggtgatgaag tcattctctt tgcaccgttt tatgattcct atgaagcaac actctctatg    420

gctggtgcta aagtaaaagg aatcacttta cgtccaccgg acttctccat ccctttggaa    480

gagcttaaag ctgcggtaac taacaagact cgagccatcc ttatgaacac tccgcacaac    540

ccgaccggga agatgttcac tagggaggag cttgaaacca ttgcatctct ctgcattgaa    600

aacgatgtgc ttgtgttctc ggatgaagta tacgataagc ttgcgtttga aatggatcac    660

atttctatag cttctcttcc cggtatgtat gaaagaactg tgaccatgaa ttccctggga    720

aagactttct ctttaaccgg atggaagatc ggctgggcga ttgcgccgcc tcatctgact    780

tggggagttc gacaagcaca ctcttacctc acattcgcca catcaacacc agcacaatgg    840

gcagccgttg cagctctcaa ggcaccagag tcttacttca aagagctgaa aagagattac    900

aatgtgaaaa aggagactct ggttaagggt ttgaaggaag tcggatttac agtgttccca    960

tcgagcggga cttactttgt ggttgctgat cacactccat ttggaatgga gaacgatgtt   1020

gctttctgtg agtatcttat tgaagaagtt ggggtcgttg cgatcccaac gagcgtcttt   1080

tatctgaatc cagaagaagg gaagaatttg gttaggtttg cgttctgtaa agacgaagag   1140

acgttgcgtg gtgcaattga gaggatgaag cagaagctta agagaaaagt ctga         1194


<210> SEQ ID NO 21
<211> LENGTH: 397
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 21

Met Ala Thr Gln Asn Glu Ser Thr Gln Lys Pro Val Gln Val Ala Lys
1               5                   10                  15

Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Ile Leu
            20                  25                  30

Ala Val Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe
        35                  40                  45

Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala Ile Lys Asp
    50                  55                  60

Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Ile Pro Gln Leu Asn Ser
65                  70                  75                  80

Ala Ile Ala Ala Arg Phe Arg Glu Asp Thr Gly Leu Val Val Asp Pro
                85                  90                  95

Glu Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala
            100                 105                 110

Ala Met Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala
        115                 120                 125

Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Lys
    130                 135                 140

Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe Ser Ile Pro Leu Glu
145                 150                 155                 160

Glu Leu Lys Ala Ala Val Thr Asn Lys Thr Arg Ala Ile Leu Met Asn
                165                 170                 175

Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Glu
            180                 185                 190

Thr Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val Phe Ser Asp
        195                 200                 205
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Glu Val Tyr Asp Lys Leu Ala Phe Glu Met Asp His Ile Ser Ile Ala
    210                 215                 220

Ser Leu Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly
225                 230                 235                 240

Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro
                245                 250                 255

Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Tyr Leu Thr Phe
            260                 265                 270

Ala Thr Ser Thr Pro Ala Gln Trp Ala Ala Val Ala Ala Leu Lys Ala
        275                 280                 285

Pro Glu Ser Tyr Phe Lys Glu Leu Lys Arg Asp Tyr Asn Val Lys Lys
    290                 295                 300

Glu Thr Leu Val Lys Gly Leu Lys Glu Val Gly Phe Thr Val Phe Pro
305                 310                 315                 320

Ser Ser Gly Thr Tyr Phe Val Val Ala Asp His Thr Pro Phe Gly Met
                325                 330                 335

Glu Asn Asp Val Ala Phe Cys Glu Tyr Leu Ile Glu Glu Val Gly Val
            340                 345                 350

Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu Glu Gly Lys
        355                 360                 365

Asn Leu Val Arg Phe Ala Phe Cys Lys Asp Glu Glu Thr Leu Arg Gly
    370                 375                 380

Ala Ile Glu Arg Met Lys Gln Lys Leu Lys Arg Lys Val
385                 390                 395


<210> SEQ ID NO 22
<211> LENGTH: 1680
<212> TYPE: DNA
<213> ORGANISM: Lycopersicon esculentum

<400> SEQUENCE: 22

ggtaccgttt gaatcctcct taaagttttt ctctggagaa actgtagtaa ttttactttg     60

ttgtgttccc ttcatctttt gaattaatgg catttgtttt aatactaatc tgcttctgaa    120

acttgtaatg tatgtatatc agtttcttat aatttatcca agtaatatct tccattctct    180

atgcaattgc ctgcataagc tcgacaaaag agtacatcaa cccctcctcc tctggactac    240

tctagctaaa cttgaatttc cccttaagat tatgaaattg atatatcctt aacaaacgac    300

tccttctgtt ggaaaatgta gtacttgtct ttcttctttt gggtatatat agtttatata    360

caccatacta tgtacaacat ccaagtagag tgaaatggat acatgtacaa gacttatttg    420

attgattgat gacttgagtt gccttaggag taacaaattc ttaggtcaat aaatcgttga    480

tttgaaatta atctctctgt cttagacaga taggaattat gacttccaat ggtccagaaa    540

gcaaagttcg cactgagggt atacttggaa ttgagacttg cacaggtcca gaaaccaaag    600

ttcccatcga gctctaaaat cacatctttg gaatgaaatt caattagaga taagttgctt    660

catagcatag gtaaaatgga agatgtgaag taacctgcaa taatcagtga aatgacatta    720

atacactaaa tacttcatat gtaattatcc tttccaggtt aacaatactc tataaagtaa    780

gaattatcag aaatgggctc atcaaacttt tgtactatgt atttcatata aggaagtata    840

actatacata agtgtataca caactttatt cctattttgt aaaggtggag agactgtttt    900

cgatggatct aaagcaatat gtctataaaa tgcattgata taataattat ctgagaaaat    960
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ccagaattgg cgttggatta tttcagccaa atagaagttt gtaccatact tgttgattcc   1020
ttctaagtta aggtgaagta tcattcataa acagttttcc ccaaagtact actcaccaag   1080
tttccctttg tagaattaac agttcaaata tatggcgcag aaattactct atgcccaaaa   1140
ccaaacgaga aagaaacaaa atacaggggt tgcagacttt attttcgtgt tagggtgtgt   1200
tttttcatgt aattaatcaa aaaatattat gacaaaaaca tttatacata tttttactca   1260
acactctggg tatcagggtg ggttgtgttc gacaatcaat atggaaagga agtattttcc   1320
ttattttttt agttaatatt ttcagttata ccaaacatac cttgtgatat tatttttaaa   1380
aatgaaaaac tcgtcagaaa gaaaaagcaa aagcaacaaa aaaattgcaa gtatttttta   1440
aaaaagaaaa aaaaaacata tcttgtttgt cagtatggga agtttgagat aaggacgagt   1500
gaggggttaa aattcagtgg ccattgattt tgtaatgcca agaaccacaa aatccaatgg   1560
ttaccattcc tgtaagatga ggtttgctaa ctctttttgt ccgttagata ggaagcctta   1620
tcactatata tacaaggcgt cctaataacc tcttagtaac caattatttc agcaccatgg   1680
```

<210> SEQ ID NO 23
<211> LENGTH: 1230
<212> TYPE: DNA
<213> ORGANISM: Phyllostachys bambusoides

<400> SEQUENCE: 23

```
atggcctccg cggccgtctc caccgtcgcc accgccgccg acggcgtcgc gaagccgacg     60
gagaagcagc cggtacaggt cgcaaagcgt ttggaaaagt ttaagacaac aattttcaca    120
cagatgagca tgcttgccat caagcatgga gcaataaacc tcggccaggg ctttccgaat    180
tttgatggcc ctgactttgt gaaagaagct gctattcaag ctatcaatgc tgggaagaat    240
cagtatgcaa gaggatatgg tgtgcctgaa ctgaactcgg ctgttgctga aaggttcctg    300
aaggacagtg gcttgcaagt cgatcccgag aaggaagtta ctgtcacatc tgggtgcacg    360
gaagcgatag ctgcaacgat attgggtctt atcaaccctg gcgatgaagt gatcttgttt    420
gctccattct atgattcata cgaggctacg ctgtcgatgg ctggtgccaa tgtaaaagcc    480
attactctcc gtcctccaga ttttgcagtc cctcttgagg agctaaaggc cacagtctct    540
aagaacacca gagcgataat gataaacaca ccacacaatc ctactgggaa aatgttttct    600
agggaagaac ttgaattcat tgctactctc tgcaagaaaa atgatgtgtt gctttttgct    660
gatgaggtct atgacaagtt ggcatttgag gcagatcata tatcaatggc ttctattcct    720
ggcatgtatg agaggactgt gactatgaac tctctgggga agacattctc tctaacagga    780
tggaagatcg gttgggcaat agcaccacca cacctgacat ggggtgtaag gcaggcacac    840
tcattcctca catttgccac ctgcacacca atgcaatcgg cggcggcggc ggctcttaga    900
gcaccagata gctactatgg ggagctgaag agggattacg gtgcaaagaa agcgatacta    960
gtcgacggac tcaaggctgc aggttttatt gtttaccctt caagtggaac atactttgtc   1020
atggtcgatc acaccccgtt tggtttcgac aatgatattg agttctgcga gtatttgatc   1080
cgcgaagtcg gtgttgtcgc cataccacca agcgtatttt atctcaaccc tgaggatggg   1140
aagaacttgg tgaggttcac cttctgcaag gatgatgata cgctgagagc cgcagttgag   1200
aggatgaaga caaagctcag gaaaaaatga                                    1230
```

<210> SEQ ID NO 24

-continued

<211> LENGTH: 409
<212> TYPE: PRT
<213> ORGANISM: Phyllostachys bambusoides

<400> SEQUENCE: 24

Met Ala Ser Ala Ala Val Ser Thr Val Ala Thr Ala Ala Asp Gly Val
1 5 10 15

Ala Lys Pro Thr Glu Lys Gln Pro Val Gln Val Ala Lys Arg Leu Glu
20 25 30

Lys Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Met Leu Ala Ile Lys
35 40 45

His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly Pro
50 55 60

Asp Phe Val Lys Glu Ala Ala Ile Gln Ala Ile Asn Ala Gly Lys Asn
65 70 75 80

Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu Leu Asn Ser Ala Val Ala
85 90 95

Glu Arg Phe Leu Lys Asp Ser Gly Leu Gln Val Asp Pro Glu Lys Glu
100 105 110

Val Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Thr Ile Leu
115 120 125

Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe Tyr
130 135 140

Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Asn Val Lys Ala
145 150 155 160

Ile Thr Leu Arg Pro Pro Asp Phe Ala Val Pro Leu Glu Glu Leu Lys
165 170 175

Ala Thr Val Ser Lys Asn Thr Arg Ala Ile Met Ile Asn Thr Pro His
180 185 190

Asn Pro Thr Gly Lys Met Phe Ser Arg Glu Glu Leu Glu Phe Ile Ala
195 200 205

Thr Leu Cys Lys Lys Asn Asp Val Leu Leu Phe Ala Asp Glu Val Tyr
210 215 220

Asp Lys Leu Ala Phe Glu Ala Asp His Ile Ser Met Ala Ser Ile Pro
225 230 235 240

Gly Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr Phe
245 250 255

Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro Pro His Leu
260 265 270

Thr Trp Gly Val Arg Gln Ala His Ser Phe Leu Thr Phe Ala Thr Cys
275 280 285

Thr Pro Met Gln Ser Ala Ala Ala Ala Ala Leu Arg Ala Pro Asp Ser
290 295 300

Tyr Tyr Gly Glu Leu Lys Arg Asp Tyr Gly Ala Lys Lys Ala Ile Leu
305 310 315 320

Val Asp Gly Leu Lys Ala Ala Gly Phe Ile Val Tyr Pro Ser Ser Gly
325 330 335

Thr Tyr Phe Val Met Val Asp His Thr Pro Phe Gly Phe Asp Asn Asp
340 345 350

Ile Glu Phe Cys Glu Tyr Leu Ile Arg Glu Val Gly Val Val Ala Ile
355 360 365

Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu Asp Gly Lys Asn Leu Val

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    370                 375                 380

Arg Phe Thr Phe Cys Lys Asp Asp Asp Thr Leu Arg Ala Ala Val Glu
385                 390                 395                 400

Arg Met Lys Thr Lys Leu Arg Lys Lys
                405


<210> SEQ ID NO 25
<211> LENGTH: 1858
<212> TYPE: DNA
<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 25

aaaaaagaaa aaaaaaacat atcttgtttg tcagtatggg aagtttgaga taaggacgag     60

tgaggggtta aaattcagtg gccattgatt ttgtaatgcc aagaaccaca aaatccaatg    120

gttaccattc ctgtaagatg aggtttgcta actctttttg tccgttagat aggaagcctt    180

atcactatat atacaaggcg tcctaataac ctcttagtaa ccaattattt cagcaccatg    240

gtagatctga gggtaaattt ctagtttttc tccttcattt tcttggttag gaccctttc    300

tctttttatt tttttgagct ttgatctttc tttaaactga tctatttttt aattgattgg    360

ttatggtgta aatattacat agctttaact gataatctga ttactttatt tcgtgtgtct    420

atgatgatga tgatagttac agaaccgacg aactagtatg aatctggccg gctttctcgc    480

cacgcccgcg accgcgaccg cgacgcggca tgagatgccg ttaaatccct cctcctccgc    540

ctccttcctc ctctcctcgc tccgccgctc gctcgtcgcg tcgctccgga aggcctcgcc    600

ggcggcggcc gcggcgctct cccccatggc ctccgcgtcc accgtcgccg ccgagaacgg    660

cgccgccaag gcggcggcgg agaagcagca gcagcagcct gtgcaggttg caaagcggtt    720

ggaaaagttt aagacgacca ttttcacaca gatgagtatg cttgccatca agcatggagc    780

aataaacctt ggccagggtt ttccgaattt cgatggccct gactttgtaa aagaggctgc    840

tattcaagct atcaatgctg ggaagaatca gtacgcaaga ggatatggtg tgcctgaact    900

gaactcagct attgctgaaa gattcctgaa ggacagcgga ctgcaagtcg atccggagaa    960

ggaagttact gtcacatctg gatgcacaga agctatagct gcaacaattt taggtctaat   1020

taatccaggc gatgaagtga tattgtttgc tccattctat gattcatatg aggctaccct   1080

gtcaatggct ggtgccaacg taaaagccat tactctccgt cctccagatt tttcagtccc   1140

tcttgaagag ctaaaggctg cagtctcgaa gaacaccaga gctattatga taaacacccc   1200

gcacaatcct actgggaaaa tgtttacaag ggaagaactt gagtttattg ccactctctg   1260

caaggaaaat gatgtgctgc tttttgctga tgaggtctac gacaagttag cttttgaggc   1320

agatcatata tcaatggctt ctattcctgg catgtatgag aggaccgtga ccatgaactc   1380

tcttgggaag acattctctc ttacaggatg gaagatcggt tgggcaatcg caccgccaca   1440

cctgacatgg ggtgtaaggc aggcacactc attcctcacg tttgcgacct gcacaccaat   1500

gcaagcagct gcagctgcag ctctgagagc accagatagc tactatgagg aactgaggag   1560

ggattatgga gctaagaagg cattgctagt caacggactc aaggatgcag gtttcattgt   1620

ctatccttca agtggaacat acttcgtcat ggtcgaccac accccatttg gtttcgacaa   1680

tgatattgag ttctgcgagt atttgattcg cgaagtcggt gttgtcgcca taccacctag   1740

tgtattttat ctcaaccctg aggatgggaa gaacttggtg aggttcacct tttgcaagga   1800
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tgatgagacg ctgagagccg cggttgagag gatgaagaca aagctcagga aaaaatga 1858

<210> SEQ ID NO 26
<211> LENGTH: 1724
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA encoding Hordeum vulgare GPT
protein

<400> SEQUENCE: 26

aaaaaagaaa aaaaaaacat atcttgtttg tcagtatggg aagtttgaga taaggacgag 60

tgaggggtta aaattcagtg gccattgatt ttgtaatgcc aagaaccaca aaatccaatg 120

gttaccattc ctgtaagatg aggtttgcta actctttttg tccgttagat aggaagcctt 180

atcactatat atacaaggcg tcctaataac ctcttagtaa ccaattattt cagcaccatg 240

gtagatctga gggtaaattt ctagtttttc tccttcattt tcttggttag gaccctttc 300

tctttttatt tttttgagct ttgatctttc tttaaactga tctatttttt aattgattgg 360

ttatggtgta aatattacat agctttaact gataatctga ttactttatt tcgtgtgtct 420

atgatgatga tgatagttac agaaccgacg aactagtatg gcatccgccc ccgcctccgc 480

ctccgcggcc ctctccaccg ccgcccccgc cgacaacggg gccgccaagc ccacggagca 540

gcggccggta caggtggcta agcgattgga gaagttcaaa acaacaattt tcacacagat 600

gagcatgctc gcagtgaagc atggagcaat aaaccttgga caggggtttc ccaattttga 660

tggccctgac tttgtcaaag atgctgctat tgaggctatc aaagctggaa agaatcagta 720

tgcaagagga tatggtgtgc ctgaattgaa ctcagctgtt gctgagagat ttctcaagga 780

cagtggattg cacatcgatc ctgataagga agttactgtt acatctgggt gcacagaagc 840

aatagctgca acgatattgg gtctgatcaa ccctggggat gaagtcatac tgtttgctcc 900

attctatgat tcttatgagg ctacactgtc catggctggt gcgaatgtca aagccattac 960

actccgccct ccggactttg cagtccctct tgaagagcta aaggctgcag tctcgaagaa 1020

taccagagca ataatgatta atacacctca caaccctacc gggaaaatgt tcacaaggga 1080

ggaacttgag ttcattgctg atctctgcaa ggaaaatgac gtgttgctct ttgccgatga 1140

ggtctacgac aagctggcgt ttgaggcgga tcacatatca atggcttcta ttcctggcat 1200

gtatgagagg accgtcacta tgaactccct ggggaagacg ttctccttga ccggatggaa 1260

gatcggctgg gcgatagcac caccgcacct gacatggggc gtaaggcagg cacactcctt 1320

cctcacattc gccacctcca cgccgatgca atcagcagcg gcggcggccc tgagagcacc 1380

ggacagctac tttgaggagc tgaagaggga ctacggcgca aagaaagcgc tgctggtgga 1440

cgggctcaag gcggcgggct tcatcgtcta cccttcgagc ggaacctact tcatcatggt 1500

cgaccacacc ccgttcgggt tcgacaacga cgtcgagttc tgcgagtact tgatccgcga 1560

ggtcggcgtc gtggccatcc cgccaagcgt gttctacctg aacccggagg acgggaagaa 1620

cctggtgagg ttcaccttct gcaaggacga cgacacgcta agggcggcgg tggacaggat 1680

gaaggccaag ctcaggaaga aatgattgag gggcgcacgt gtga 1724

<210> SEQ ID NO 27
<211> LENGTH: 1868
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA encoding Arabidopsis thaliana GPT protein

<400> SEQUENCE: 27

catggagtca aagattcaaa tagaggacct aacagaactc gccgtaaaga ctggcgaaca 60

gttcatacag agtctcttac gactcaatga caagaagaaa atcttcgtca acatggtgga 120

gcacgacaca cttgtctact ccaaaaatat caaagataca gtctcagaag accaaagggc 180

aattgagact tttcaacaaa gggtaatatc cggaaacctc ctcggattcc attgcccagc 240

tatctgtcac tttattgtga agatagtgga aaaggaaggt ggctcctaca aatgccatca 300

ttgcgataaa ggaaaggcca tcgttgaaga tgcctctgcc gacagtggtc ccaaagatgg 360

acccccaccc acgaggagca tcgtggaaaa agaagacgtt ccaaccacgt cttcaaagca 420

agtggattga tgtgatatct ccactgacgt aagggatgac gcacaatccc actatccttc 480

gcaagaccct tcctctatat aaggaagttc atttcatttg gagagaacac gggggactct 540

tgaccatgta cctggacata aatggtgtga tgatcaaaca gtttagcttc aaagcctctc 600

ttctcccatt ctcttctaat ttccgacaaa gctccgccaa aatccatcgt cctatcggag 660

ccaccatgac cacagtttcg actcagaacg agtctactca aaaacccgtc caggtggcga 720

agagattaga gaagttcaag actactattt tcactcaaat gagcatattg gcagttaaac 780

atggagcgat caatttaggc caaggctttc ccaatttcga cggtcctgat tttgttaaag 840

aagctgcgat ccaagctatt aaagatggta aaaaccagta tgctcgtgga tacggcattc 900

ctcagctcaa ctctgctata gctgcgcggt ttcgtgaaga tacgggtctt gttgttgatc 960

ctgagaaaga agttactgtt acatctggtt gcacagaagc catagctgca gctatgttgg 1020

gtttaataaa ccctggtgat gaagtcattc tctttgcacc gttttatgat tcctatgaag 1080

caacactctc tatggctggt gctaaagtaa aaggaatcac tttacgtcca ccggacttct 1140

ccatcccttt ggaagagctt aaagctgcgg taactaacaa gactcgagcc atccttatga 1200

acactccgca caacccgacc gggaagatgt tcactaggga ggagcttgaa accattgcat 1260

ctctctgcat tgaaaacgat gtgcttgtgt tctcggatga agtatacgat aagcttgcgt 1320

ttgaaatgga tcacatttct atagcttctc ttcccggtat gtatgaaaga actgtgacca 1380

tgaattccct gggaaagact ttctctttaa ccggatggaa gatcggctgg gcgattgcgc 1440

cgcctcatct gacttgggga gttcgacaag cacactctta cctcacattc gccacatcaa 1500

caccagcaca atgggcagcc gttgcagctc tcaaggcacc agagtcttac ttcaaagagc 1560

tgaaaagaga ttacaatgtg aaaaaggaga ctctggttaa gggtttgaag gaagtcggat 1620

ttacagtgtt cccatcgagc gggacttact ttgtggttgc tgatcacact ccatttggaa 1680

tggagaacga tgttgctttc tgtgagtatc ttattgaaga agttggggtc gttgcgatcc 1740

caacgagcgt cttttatctg aatccagaag aagggaagaa tttggttagg tttgcgttct 1800

gtaaagacga agagacgttg cgtggtgcaa ttgagaggat gaagcagaag cttaagagaa 1860

aagtctga 1868

<210> SEQ ID NO 28
<211> LENGTH: 1780
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic DNA encoding Arabidopsis thaliana GPT protein

<400> SEQUENCE: 28

aaaaaagaaa aaaaaaacat atcttgtttg tcagtatggg aagtttgaga taaggacgag 60

tgaggggtta aaattcagtg gccattgatt ttgtaatgcc aagaaccaca aaatccaatg 120

gttaccattc ctgtaagatg aggtttgcta actctttttg tccgttagat aggaagcctt 180

atcactatat atacaaggcg tcctaataac ctcttagtaa ccaattattt cagcaccatg 240

gtagatctga gggtaaattt ctagtttttc tccttcattt tcttggttag gaccctttc 300

tctttttatt tttttgagct ttgatctttc tttaaactga tctatttttt aattgattgg 360

ttatggtgta aatattacat agctttaact gataatctga ttactttatt tcgtgtgtct 420

atgatgatga tgatagttac agaaccgacg aactagtatg tacctggaca taaatggtgt 480

gatgatcaaa cagtttagct tcaaagcctc tcttctccca ttctcttcta atttccgaca 540

aagctccgcc aaaatccatc gtcctatcgg agccaccatg accacagttt cgactcagaa 600

cgagtctact caaaaacccg tccaggtggc gaagagatta gagaagttca agactactat 660

tttcactcaa atgagcatat tggcagttaa acatggagcg atcaatttag gccaaggctt 720

tcccaatttc gacggtcctg attttgttaa agaagctgcg atccaagcta ttaaagatgg 780

taaaaaccag tatgctcgtg gatacggcat tcctcagctc aactctgcta tagctgcgcg 840

gtttcgtgaa gatacgggtc ttgttgttga tcctgagaaa gaagttactg ttacatctgg 900

ttgcacagaa gccatagctg cagctatgtt gggtttaata aaccctggtg atgaagtcat 960

tctctttgca ccgttttatg attcctatga agcaacactc tctatggctg gtgctaaagt 1020

aaaaggaatc actttacgtc caccggactt ctccatccct ttggaagagc ttaaagctgc 1080

ggtaactaac aagactcgag ccatccttat gaacactccg cacaacccga ccgggaagat 1140

gttcactagg gaggagcttg aaaccattgc atctctctgc attgaaaacg atgtgcttgt 1200

gttctcggat gaagtatacg ataagcttgc gtttgaaatg gatcacattt ctatagcttc 1260

tcttcccggt atgtatgaaa gaactgtgac catgaattcc ctgggaaaga ctttctcttt 1320

aaccggatgg aagatcggct gggcgattgc gccgcctcat ctgacttggg gagttcgaca 1380

agcacactct tacctcacat tcgccacatc aacaccagca caatgggcag ccgttgcagc 1440

tctcaaggca ccagagtctt acttcaaaga gctgaaaaga gattacaatg tgaaaaagga 1500

gactctggtt aagggtttga aggaagtcgg atttacagtg ttcccatcga gcgggactta 1560

ctttgtggtt gctgatcaca ctccatttgg aatggagaac gatgttgctt tctgtgagta 1620

tcttattgaa gaagttgggg tcgttgcgat cccaacgagc gtcttttatc tgaatccaga 1680

agaagggaag aatttggtta ggtttgcgtt ctgtaaagac gaagagacgt tgcgtggtgc 1740

aattgagagg atgaagcaga agcttaagag aaaagtctga 1780

<210> SEQ ID NO 29
<211> LENGTH: 1155
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 29

gtggcgaaga gattagagaa gttcaagact actattttca ctcaaatgag catattggca 60

gttaaacatg gagcgatcaa tttaggccaa ggctttccca atttcgacgg tcctgatttt 120

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gttaaagaag ctgcgatcca agctattaaa gatggtaaaa accagtatgc tcgtggatac    180

ggcattcctc agctcaactc tgctatagct gcgcggtttc gtgaagatac gggtcttgtt    240

gttgatcctg agaaagaagt tactgttaca tctggttgca cagaagccat agctgcagct    300

atgttgggtt taataaaccc tggtgatgaa gtcattctct ttgcaccgtt ttatgattcc    360

tatgaagcaa cactctctat ggctggtgct aaagtaaaag gaatcacttt acgtccaccg    420

gacttctcca tccctttgga agagcttaaa gctgcggtaa ctaacaagac tcgagccatc    480

cttatgaaca ctccgcacaa cccgaccggg aagatgttca ctagggagga gcttgaaacc    540

attgcatctc tctgcattga aaacgatgtg cttgtgttct cggatgaagt atacgataag    600

cttgcgtttg aaatggatca catttctata gcttctcttc ccggtatgta tgaaagaact    660

gtgaccatga attccctggg aaagactttc tctttaaccg gatggaagat cggctgggcg    720

attgcgccgc ctcatctgac ttggggagtt cgacaagcac actcttacct cacattcgcc    780

acatcaacac cagcacaatg ggcagccgtt gcagctctca aggcaccaga gtcttacttc    840

aaagagctga aaagagatta caatgtgaaa aaggagactc tggttaaggg tttgaaggaa    900

gtcggattta cagtgttccc atcgagcggg acttactttg tggttgctga tcacactcca    960

tttggaatgg agaacgatgt tgctttctgt gagtatctta ttgaagaagt tggggtcgtt   1020

gcgatcccaa cgagcgtctt ttatctgaat ccagaagaag ggaagaattt ggttaggttt   1080

gcgttctgta aagacgaaga gacgttgcgt ggtgcaattg agaggatgaa gcagaagctt   1140

aagagaaaag tctga                                                    1155
```

<210> SEQ ID NO 30
<211> LENGTH: 384
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 30

```
Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Ile Leu Ala Val Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala
        35                  40                  45

Ile Lys Asp Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Ile Pro Gln
    50                  55                  60

Leu Asn Ser Ala Ile Ala Ala Arg Phe Arg Glu Asp Thr Gly Leu Val
65                  70                  75                  80

Val Asp Pro Glu Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Ala Met Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125

Gly Ala Lys Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe Ser Ile
    130                 135                 140

Pro Leu Glu Glu Leu Lys Ala Ala Val Thr Asn Lys Thr Arg Ala Ile
145                 150                 155                 160

Leu Met Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
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                165                 170                 175

Glu Leu Glu Thr Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val
            180                 185                 190

Phe Ser Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Met Asp His Ile
        195                 200                 205

Ser Ile Ala Ser Leu Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn
    210                 215                 220

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
225                 230                 235                 240

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

Leu Thr Phe Ala Thr Ser Thr Pro Ala Gln Trp Ala Ala Val Ala Ala
            260                 265                 270

Leu Lys Ala Pro Glu Ser Tyr Phe Lys Glu Leu Lys Arg Asp Tyr Asn
        275                 280                 285

Val Lys Lys Glu Thr Leu Val Lys Gly Leu Lys Glu Val Gly Phe Thr
    290                 295                 300

Val Phe Pro Ser Ser Gly Thr Tyr Phe Val Val Ala Asp His Thr Pro
305                 310                 315                 320

Phe Gly Met Glu Asn Asp Val Ala Phe Cys Glu Tyr Leu Ile Glu Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu
            340                 345                 350

Glu Gly Lys Asn Leu Val Arg Phe Ala Phe Cys Lys Asp Glu Glu Thr
        355                 360                 365

Leu Arg Gly Ala Ile Glu Arg Met Lys Gln Lys Leu Lys Arg Lys Val
    370                 375                 380


<210> SEQ ID NO 31
<211> LENGTH: 384
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 31

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Glu Phe Val Lys Glu Ala Ala Ile Gln Ala
        35                  40                  45

Ile Lys Asp Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Asp
    50                  55                  60

Leu Asn Ser Ala Val Ala Asp Arg Phe Lys Lys Asp Thr Gly Leu Val
65                  70                  75                  80

Val Asp Pro Glu Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Thr Met Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125

Gly Ala Gln Ile Lys Ser Ile Thr Leu Arg Pro Pro Asp Phe Ala Val
    130                 135                 140
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Pro Met Asp Glu Leu Lys Ser Ala Ile Ser Lys Asn Thr Arg Ala Ile
145                 150                 155                 160

Leu Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
                165                 170                 175

Glu Leu Asn Val Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val
            180                 185                 190

Phe Thr Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Met Asp His Ile
        195                 200                 205

Ser Met Ala Ser Leu Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn
    210                 215                 220

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Thr
225                 230                 235                 240

Val Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe
                245                 250                 255

Leu Thr Phe Ala Thr Cys Thr Pro Met Gln Trp Ala Ala Ala Thr Ala
            260                 265                 270

Leu Arg Ala Pro Asp Ser Tyr Tyr Glu Glu Leu Lys Arg Asp Tyr Ser
        275                 280                 285

Ala Lys Lys Ala Ile Leu Val Glu Gly Leu Lys Ala Val Gly Phe Arg
    290                 295                 300

Val Tyr Pro Ser Ser Gly Thr Tyr Phe Val Val Val Asp His Thr Pro
305                 310                 315                 320

Phe Gly Leu Lys Asp Asp Ile Ala Phe Cys Glu Tyr Leu Ile Lys Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu His Pro Glu
            340                 345                 350

Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Glu Gly Thr
        355                 360                 365

Leu Arg Ala Ala Val Glu Arg Met Lys Glu Lys Leu Lys Pro Lys Gln
    370                 375                 380


<210> SEQ ID NO 32
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

<400> SEQUENCE: 32

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala
        35                  40                  45

Ile Asn Ala Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu
    50                  55                  60

Leu Asn Ser Ala Ile Ala Glu Arg Phe Leu Lys Asp Ser Gly Leu Gln
65                  70                  75                  80

Val Asp Pro Glu Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Thr Ile Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125
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Gly Ala Asn Val Lys Ala Ile Thr Leu Arg Pro Pro Asp Phe Ser Val
    130                 135                 140

Pro Leu Glu Glu Leu Lys Ala Ala Val Ser Lys Asn Thr Arg Ala Ile
145                 150                 155                 160

Met Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
                165                 170                 175

Glu Leu Glu Phe Ile Ala Thr Leu Cys Lys Glu Asn Asp Val Leu Leu
            180                 185                 190

Phe Ala Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Ala Asp His Ile
        195                 200                 205

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

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
225                 230                 235                 240

Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe
                245                 250                 255

Leu Thr Phe Ala Thr Cys Thr Pro Met Gln Ala Ala Ala Ala Ala Ala
            260                 265                 270

Leu Arg Ala Pro Asp Ser Tyr Tyr Glu Glu Leu Arg Arg Asp Tyr Gly
        275                 280                 285

Ala Lys Lys Ala Leu Leu Val Asn Gly Leu Lys Asp Ala Gly Phe Ile
    290                 295                 300

Val Tyr Pro Ser Ser Gly Thr Tyr Phe Val Met Val Asp His Thr Pro
305                 310                 315                 320

Phe Gly Phe Asp Asn Asp Ile Glu Phe Cys Glu Tyr Leu Ile Arg Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu
            340                 345                 350

Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Asp Glu Thr
        355                 360                 365

Leu Arg Ala Ala Val Glu Arg Met Lys Thr Lys Leu Arg Lys Lys
    370                 375                 380


<210> SEQ ID NO 33
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 33

Ala Lys Arg Leu Glu Lys Phe Gln Thr Thr Ile Phe Thr Gln Met Ser
1               5                   10                  15

Leu Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro
            20                  25                  30

Asn Phe Asp Gly Pro Glu Phe Val Lys Glu Ala Ala Ile Gln Ala Ile
        35                  40                  45

Arg Asp Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Asp Leu
    50                  55                  60

Asn Ile Ala Ile Ala Glu Arg Phe Lys Lys Asp Thr Gly Leu Val Val
65                  70                  75                  80

Asp Pro Glu Lys Glu Ile Thr Val Thr Ser Gly Cys Thr Glu Ala Ile
                85                  90                  95

Ala Ala Thr Met Ile Gly Leu Ile Asn Pro Gly Asp Glu Val Ile Met
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            100                 105                 110

Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly
        115                 120                 125

Ala Lys Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe Ala Val Pro
    130                 135                 140

Leu Glu Glu Leu Lys Ser Thr Ile Ser Lys Asn Thr Arg Ala Ile Leu
145                 150                 155                 160

Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu Glu
                165                 170                 175

Leu Asn Cys Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val Phe
            180                 185                 190

Thr Asp Glu Val Tyr Asp Lys Leu Ala Phe Asp Met Glu His Ile Ser
        195                 200                 205

Met Ala Ser Leu Pro Gly Met Phe Glu Arg Thr Val Thr Leu Asn Ser
    210                 215                 220

Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile
225                 230                 235                 240

Ala Pro Pro His Leu Ser Trp Gly Val Arg Gln Ala His Ala Phe Leu
                245                 250                 255

Thr Phe Ala Thr Ala His Pro Phe Gln Cys Ala Ala Ala Ala Ala Leu
            260                 265                 270

Arg Ala Pro Asp Ser Tyr Tyr Val Glu Leu Lys Arg Asp Tyr Met Ala
        275                 280                 285

Lys Arg Ala Ile Leu Ile Glu Gly Leu Lys Ala Val Gly Phe Lys Val
    290                 295                 300

Phe Pro Ser Ser Gly Thr Tyr Phe Val Val Val Asp His Thr Pro Phe
305                 310                 315                 320

Gly Leu Glu Asn Asp Val Ala Phe Cys Glu Tyr Leu Val Lys Glu Val
                325                 330                 335

Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu Glu
            340                 345                 350

Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Glu Glu Thr Ile
        355                 360                 365

Arg Ser Ala Val Glu Arg Met Lys Ala Lys Leu Arg Lys Val Asp
    370                 375                 380


<210> SEQ ID NO 34
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 34

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Met Leu Ala Val Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Asp Ala Ala Ile Glu Ala
        35                  40                  45

Ile Lys Ala Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu
    50                  55                  60

Leu Asn Ser Ala Val Ala Glu Arg Phe Leu Lys Asp Ser Gly Leu His
65                  70                  75                  80
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Ile Asp Pro Asp Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Thr Ile Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125

Gly Ala Asn Val Lys Ala Ile Thr Leu Arg Pro Pro Asp Phe Ala Val
    130                 135                 140

Pro Leu Glu Glu Leu Lys Ala Ala Val Ser Lys Asn Thr Arg Ala Ile
145                 150                 155                 160

Met Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
                165                 170                 175

Glu Leu Glu Phe Ile Ala Asp Leu Cys Lys Glu Asn Asp Val Leu Leu
            180                 185                 190

Phe Ala Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Ala Asp His Ile
        195                 200                 205

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

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
225                 230                 235                 240

Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe
                245                 250                 255

Leu Thr Phe Ala Thr Ser Thr Pro Met Gln Ser Ala Ala Ala Ala Ala
            260                 265                 270

Leu Arg Ala Pro Asp Ser Tyr Phe Glu Glu Leu Lys Arg Asp Tyr Gly
        275                 280                 285

Ala Lys Lys Ala Leu Leu Val Asp Gly Leu Lys Ala Ala Gly Phe Ile
    290                 295                 300

Val Tyr Pro Ser Ser Gly Thr Tyr Phe Ile Met Val Asp His Thr Pro
305                 310                 315                 320

Phe Gly Phe Asp Asn Asp Val Glu Phe Cys Glu Tyr Leu Ile Arg Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu
            340                 345                 350

Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Asp Asp Thr
        355                 360                 365

Leu Arg Ala Ala Val Asp Arg Met Lys Ala Lys Leu Arg Lys Lys
    370                 375                 380


<210> SEQ ID NO 35
<211> LENGTH: 382
<212> TYPE: PRT
<213> ORGANISM: Danio rerio

<400> SEQUENCE: 35

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala
        35                  40                  45

Ile Arg Asp Gly Asn Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Asp
    50                  55                  60
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Leu Asn Ile Ala Ile Ser Glu Arg Tyr Lys Lys Asp Thr Gly Leu Ala
65                  70                  75                  80

Val Asp Pro Glu Lys Glu Ile Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Thr Val Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Val Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125

Gly Ala Lys Val Lys Gly Ile Thr Leu Arg Pro Pro Asp Phe Ala Leu
    130                 135                 140

Pro Ile Glu Glu Leu Lys Ser Thr Ile Ser Lys Asn Thr Arg Ala Ile
145                 150                 155                 160

Leu Leu Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Pro Glu
                165                 170                 175

Glu Leu Asn Thr Ile Ala Ser Leu Cys Ile Glu Asn Asp Val Leu Val
            180                 185                 190

Phe Ser Asp Glu Val Tyr Asp Lys Leu Ala Phe Asp Met Glu His Ile
        195                 200                 205

Ser Ile Ala Ser Leu Pro Gly Met Phe Glu Arg Thr Val Thr Met Asn
    210                 215                 220

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
225                 230                 235                 240

Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ala Phe
                245                 250                 255

Leu Thr Phe Ala Thr Ser Asn Pro Met Gln Trp Ala Ala Ala Val Ala
            260                 265                 270

Leu Arg Ala Pro Asp Ser Tyr Tyr Thr Glu Leu Lys Arg Asp Tyr Met
        275                 280                 285

Ala Lys Arg Ser Ile Leu Val Glu Gly Leu Lys Ala Val Gly Phe Lys
    290                 295                 300

Val Phe Pro Ser Ser Gly Thr Tyr Phe Val Val Val Asp His Thr Pro
305                 310                 315                 320

Phe Gly His Glu Asn Asp Ile Ala Phe Cys Glu Tyr Leu Val Lys Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Thr Ser Val Phe Tyr Leu Asn Pro Glu
            340                 345                 350

Glu Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Glu Gly Thr
        355                 360                 365

Leu Arg Ala Ala Val Asp Arg Met Lys Glu Lys Leu Arg Lys
    370                 375                 380


<210> SEQ ID NO 36
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Phyllostachys bambusoides

<400> SEQUENCE: 36

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
1               5                   10                  15

Ser Met Leu Ala Ile Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
            20                  25                  30

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Glu Ala Ala Ile Gln Ala
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        35                  40                  45

Ile Asn Ala Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu
    50                  55                  60

Leu Asn Ser Ala Val Ala Glu Arg Phe Leu Lys Asp Ser Gly Leu Gln
65                  70                  75                  80

Val Asp Pro Glu Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
                85                  90                  95

Ile Ala Ala Thr Ile Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
            100                 105                 110

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
        115                 120                 125

Gly Ala Asn Val Lys Ala Ile Thr Leu Arg Pro Pro Asp Phe Ala Val
    130                 135                 140

Pro Leu Glu Glu Leu Lys Ala Thr Val Ser Lys Asn Thr Arg Ala Ile
145                 150                 155                 160

Met Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Ser Arg Glu
                165                 170                 175

Glu Leu Glu Phe Ile Ala Thr Leu Cys Lys Lys Asn Asp Val Leu Leu
            180                 185                 190

Phe Ala Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Ala Asp His Ile
        195                 200                 205

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

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
225                 230                 235                 240

Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe
                245                 250                 255

Leu Thr Phe Ala Thr Cys Thr Pro Met Gln Ser Ala Ala Ala Ala Ala
            260                 265                 270

Leu Arg Ala Pro Asp Ser Tyr Tyr Gly Glu Leu Lys Arg Asp Tyr Gly
        275                 280                 285

Ala Lys Lys Ala Ile Leu Val Asp Gly Leu Lys Ala Ala Gly Phe Ile
    290                 295                 300

Val Tyr Pro Ser Ser Gly Thr Tyr Phe Val Met Val Asp His Thr Pro
305                 310                 315                 320

Phe Gly Phe Asp Asn Asp Ile Glu Phe Cys Glu Tyr Leu Ile Arg Glu
                325                 330                 335

Val Gly Val Val Ala Ile Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu
            340                 345                 350

Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Asp Asp Thr
        355                 360                 365

Leu Arg Ala Ala Val Glu Arg Met Lys Thr Lys Leu Arg Lys Lys
    370                 375                 380


<210> SEQ ID NO 37
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer sequence

<400> SEQUENCE: 37

cccatcgatg tacctggaca taaatggtgt gatg                                34
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<210> SEQ ID NO 38
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer sequence

<400> SEQUENCE: 38

gatggtacct cagacttttc tcttaagctt ctgcttc 37

<210> SEQ ID NO 39
<211> LENGTH: 2992
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic expression cassette

<400> SEQUENCE: 39

ctgcagcaaa gaaacgttat tagttggtgc ttttggtggt aggaatgtag ttttctgaca 60

aagtcaatta ctgaatataa aaaaaatctg cacagctctg cgtcaacagt tgtccaaggg 120

atgcctcaaa aatctgtgca gattatcagt cgtcacgcag aagcagaaca tcatggtgtg 180

ctaggtcagc ttcttgcatt gggccatgaa tccggttggt tgttaatctc tcctctctta 240

ttctcttata ttaagatgca taactctttt atgtagtcta aaaaaaaatc cagtggatcg 300

gatagtagta cgtcatggtg ccattaggta ccgttgaacc taacagatat ttatgcatgt 360

gtatatatat agctatatag acaaaattga tgccgattat agacccaaaa gcaataggta 420

tatataatat aatacagacc acaccaccaa actaagaatc gatcaaatag acaaggcatg 480

tctccaaatt gtcttaaact atttccgtag gttcagccgt tcaggagtcg aatcagcctc 540

tgccggcgtt ttctttgcac gtacgacgga cacacatggg cataccatat agctggtcca 600

tgacattagg agagagaacg tacgtgttga cctgtagctg agatataaca aggttgatta 660

taatatcacc aaacatgaaa tcatccaagg atgacccata actatcacta ctatagtact 720

gcatctggta aaagaaattg tatagactct atttcgagca ctaccacata acgcctgcaa 780

tgtgacaccc tacctattca ctaatgtgcc tcttcccaca cgctttccac ccgtactgct 840

cacagcttta agaaccagaa caaatgagta atattagtgt cggttcatgg ctaaaaccag 900

cactgatgta catgaccaca tatgtcaaat gctgcttcta ggcatgaccc gctcttacta 960

atacctactc atcgctagaa gaattttcgg ctgataaatt ttcaatttaa gcaagagtta 1020

tctgcgttgg ttcataactc aaactgatgg ccccaaccat attagtgcaa atttcacata 1080

tgatcataac cttttcatat gaaatcggat cgagatgaac tttatataaa cattgtagct 1140

gtcgatgata cctacaattt tatagttcac aaccttttta tttcaagtca tttaaatgcc 1200

caaataggtg tttcaaatct cagatagaaa tgttcaaaag taaaaaaggt ccctatcata 1260

acataattga tatgtaagtg agttggaaaa agataagtac gtgtgagaga gatcggggat 1320

caaattctgg tgtaataatg tatgtatttc agtcataaaa attggtagca gtagttgggg 1380

ctctgtatat ataccggtaa ggatgggatg gtagtagaat aattcttttt ttgtttttag 1440

ttttttctgg tccaaaattt caaatttgga tcccttactt gtaccaacta atattaatga 1500

gtgttgaggg tagtagaggt gcaactttac cataatccct ctgtttcagg ttataagacg 1560

ttttgacttt aaatttgacc aagtttatgc gcaaatatag taatatttat aatactatat 1620

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tagtttcatt aaataaataa ttgaatatat tttcataata aatttgtgtt gagttcaaaa   1680
tattattaat tttttctaca aacttggtca aacttgaagc agtttgactt tgaccaaagt   1740
caaaacgtct tataacttga aacggatgga ttactttttt tgtggggaca agtttacaat   1800
gtttaataaa gcacaatcca tcttaatgtt ttcaagctga atattgtaaa attcatggat   1860
aaaccagctt ctaaatgttt aaccgggaaa atgtcgaacg acaaattaat atttttaagt   1920
gatggggagt attaattaag gagtgacaac tcaactttca atatcgtact aaactgtggg   1980
atttattttc taaaatttta taccctgcca attcacgtgt tgtagatctt tttttttcac   2040
taaccgacac caggtatatc aattttattg aatatagcag caaaaagaat gtgttgtact   2100
tgtaaacaaa aagcaaactg tacataaaaa aaaatgcact cctatataat taagctcata   2160
aagatgcttt gcttcgtgag ggcccaagtt ttgatgacct tttgcttgat ctcgaaatta   2220
aaatttaagt actgttaagg gagttcacac caccatcaat tttcagcctg aagaaacagt   2280
taaacaacga ccccgatgac cagtctactg ctctccacat actagctgca ttattgatca   2340
caaaacaaaa caaaacgaaa taaaaatcag cagcgagagt gtgcagagag agacaaaggt   2400
gatctggcgt ggatatctcc ccatccatcc tcacccgcgc tgcccatcac tcgccgccgc   2460
atactccatc atgtggagag aggaagacga ggaccacagc cagagcccgg gtcgagatgc   2520
caccacggcc acaacccacg agcccggcgc gacaccaccg cgcgcgcgtg agccagccac   2580
aaacgcccgc ggataggcgc gcgcacgccg gccaatccta ccacatcccc ggcctccgcg   2640
gctcgcgagc gccgctgcca tccgatccgc tgagttttgg ctatttatac gtaccgcggg   2700
agcctgtgtg cagagcagtg catctcaaga agtactcgag caaagaagga gagagcttgg   2760
tgagctgcag ccatggtaga tctgagggta aatttctagt ttttctcctt cattttcttg   2820
gttaggaccc ttttctcttt ttattttttt gagctttgat ctttctttaa actgatctat   2880
tttttaattg attggttatg gtgtaaatat tacatagctt taactgataa tctgattact   2940
ttatttcgtg tgtctatgat gatgatgata gttacagaac cgacgaacta gt           2992
```

<210> SEQ ID NO 40
<211> LENGTH: 1281
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 40

```
gcgcaggcgg ttgtgcaggc gatgcagtgc caggtggggg tgaggggcag gacggccgtc     60
ccggcgaggc agcccgcggg cagggtgtgg ggcgtcagga gggccgcccg cgccacctcc    120
gggttcaagg tgctggcgct cggcccggag accaccgggg tcatccagag gatgcagcag    180
ctgctcgaca tggacaccac gcccttcacc gacaagatca tcgccgagta catctgggtt    240
ggaggatctg gaattgacct cagaagcaaa tcaaggacga tttcgaagcc agtggaggac    300
ccgtcagagc tgccgaaatg gaactacgac ggatcgagca cggggcaggc tcctggggaa    360
gacagtgaag tcatcctata cccacaggcc atattcaagg acccattccg aggaggcaac    420
aacatactgg ttatctgtga cacctacaca ccacaggggg aacccatccc tactaacaaa    480
cgccacatgg ctgcacaaat cttcagtgac cccaaggtca cttcacaagt gccatggttc    540
ggaatcgaac aggagtacac tctgatgcag agggatgtga actggcctct tggctggcct    600
gttggagggt accctggccc ccagggtcca tactactgcg ccgtaggatc agacaagtca    660
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tttggccgtg acatatcaga tgctcactac aaggcgtgcc tttacgctgg aattgaaatc    720

agtggaacaa acggggaggt catgcctggt cagtgggagt accaggttgg acccagcgtt    780

ggtattgatg caggagacca catatgggct tccagataca ttctcgagag aatcacggag    840

caagctggtg tggtgctcac ccttgaccca aaaccaatcc agggtgactg gaacggagct    900

ggctgccaca caaactacag cacattgagc atgcgcgagg atggaggttt cgacgtgatc    960

aagaaggcaa tcctgaacct ttcacttcgc catgacttgc acatagccgc atatggtgaa   1020

ggaaacgagc ggaggttgac agggctacac gagacagcta gcatatcaga cttctcatgg   1080

ggtgtggcga accgtggctg ctctattcgt gtggggcgag acaccgaggc gaagggcaaa   1140

ggatacctgg aggaccgtcg cccggcctcc aacatggacc cgtacaccgt gacggcgctg   1200

ctggccgaga ccacgatcct gtgggagccg accctcgagg cggaggccct cgctgccaag   1260

aagctggcgc tgaaggtatg a                                             1281


<210> SEQ ID NO 41
<211> LENGTH: 426
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 41

Ala Gln Ala Val Val Gln Ala Met Gln Cys Gln Val Gly Val Arg Gly
1               5                   10                  15

Arg Thr Ala Val Pro Ala Arg Gln Pro Ala Gly Arg Val Trp Gly Val
            20                  25                  30

Arg Arg Ala Ala Arg Ala Thr Ser Gly Phe Lys Val Leu Ala Leu Gly
        35                  40                  45

Pro Glu Thr Thr Gly Val Ile Gln Arg Met Gln Gln Leu Leu Asp Met
    50                  55                  60

Asp Thr Thr Pro Phe Thr Asp Lys Ile Ile Ala Glu Tyr Ile Trp Val
65                  70                  75                  80

Gly Gly Ser Gly Ile Asp Leu Arg Ser Lys Ser Arg Thr Ile Ser Lys
                85                  90                  95

Pro Val Glu Asp Pro Ser Glu Leu Pro Lys Trp Asn Tyr Asp Gly Ser
            100                 105                 110

Ser Thr Gly Gln Ala Pro Gly Glu Asp Ser Glu Val Ile Leu Tyr Pro
        115                 120                 125

Gln Ala Ile Phe Lys Asp Pro Phe Arg Gly Gly Asn Asn Ile Leu Val
    130                 135                 140

Ile Cys Asp Thr Tyr Thr Pro Gln Gly Glu Pro Ile Pro Thr Asn Lys
145                 150                 155                 160

Arg His Met Ala Ala Gln Ile Phe Ser Asp Pro Lys Val Thr Ser Gln
                165                 170                 175

Val Pro Trp Phe Gly Ile Glu Gln Glu Tyr Thr Leu Met Gln Arg Asp
            180                 185                 190

Val Asn Trp Pro Leu Gly Trp Pro Val Gly Gly Tyr Pro Gly Pro Gln
        195                 200                 205

Gly Pro Tyr Tyr Cys Ala Val Gly Ser Asp Lys Ser Phe Gly Arg Asp
    210                 215                 220

Ile Ser Asp Ala His Tyr Lys Ala Cys Leu Tyr Ala Gly Ile Glu Ile
225                 230                 235                 240
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Ser Gly Thr Asn Gly Glu Val Met Pro Gly Gln Trp Glu Tyr Gln Val
                245                 250                 255

Gly Pro Ser Val Gly Ile Asp Ala Gly Asp His Ile Trp Ala Ser Arg
            260                 265                 270

Tyr Ile Leu Glu Arg Ile Thr Glu Gln Ala Gly Val Val Leu Thr Leu
        275                 280                 285

Asp Pro Lys Pro Ile Gln Gly Asp Trp Asn Gly Ala Gly Cys His Thr
    290                 295                 300

Asn Tyr Ser Thr Leu Ser Met Arg Glu Asp Gly Gly Phe Asp Val Ile
305                 310                 315                 320

Lys Lys Ala Ile Leu Asn Leu Ser Leu Arg His Asp Leu His Ile Ala
                325                 330                 335

Ala Tyr Gly Glu Gly Asn Glu Arg Arg Leu Thr Gly Leu His Glu Thr
            340                 345                 350

Ala Ser Ile Ser Asp Phe Ser Trp Gly Val Ala Asn Arg Gly Cys Ser
        355                 360                 365

Ile Arg Val Gly Arg Asp Thr Glu Ala Lys Gly Lys Gly Tyr Leu Glu
    370                 375                 380

Asp Arg Arg Pro Ala Ser Asn Met Asp Pro Tyr Thr Val Thr Ala Leu
385                 390                 395                 400

Leu Ala Glu Thr Thr Ile Leu Trp Glu Pro Thr Leu Glu Ala Glu Ala
                405                 410                 415

Leu Ala Ala Lys Lys Leu Ala Leu Lys Val
            420                 425


<210> SEQ ID NO 42
<211> LENGTH: 4273
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic expression cassette

<400> SEQUENCE: 42

ctgcagcaaa gaaacgttat tagttggtgc ttttggtggt aggaatgtag ttttctgaca     60

aagtcaatta ctgaatataa aaaaaatctg cacagctctg cgtcaacagt tgtccaaggg    120

atgcctcaaa aatctgtgca gattatcagt cgtcacgcag aagcagaaca tcatggtgtg    180

ctaggtcagc ttcttgcatt gggccatgaa tccggttggt tgttaatctc tcctctctta    240

ttctcttata ttaagatgca taactctttt atgtagtcta aaaaaaaatc cagtggatcg    300

gatagtagta cgtcatggtg ccattaggta ccgttgaacc taacagatat ttatgcatgt    360

gtatatatat agctatatag acaaaattga tgccgattat agacccaaaa gcaataggta    420

tatataatat aatacagacc acaccaccaa actaagaatc gatcaaatag acaaggcatg    480

tctccaaatt gtcttaaact atttccgtag gttcagccgt tcaggagtcg aatcagcctc    540

tgccggcgtt ttctttgcac gtacgacgga cacacatggg cataccatat agctggtcca    600

tgacattagg agagagaacg tacgtgttga cctgtagctg agatataaca aggttgatta    660

taatatcacc aaacatgaaa tcatccaagg atgacccata actatcacta ctatagtact    720

gcatctggta aaagaaattg tatagactct atttcgagca ctaccacata acgcctgcaa    780

tgtgacaccc tacctattca ctaatgtgcc tcttcccaca cgctttccac ccgtactgct    840

cacagcttta agaaccagaa caaatgagta atattagtgt cggttcatgg ctaaaaccag    900
```

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```
cactgatgta catgaccaca tatgtcaaat gctgcttcta ggcatgaccc gctcttacta    960
atacctactc atcgctagaa gaattttcgg ctgataaatt ttcaatttaa gcaagagtta   1020
tctgcgttgg ttcataactc aaactgatgg ccccaaccat attagtgcaa atttcacata   1080
tgatcataac cttttcatat gaaatcggat cgagatgaac tttatataaa cattgtagct   1140
gtcgatgata cctacaattt tatagttcac aaccttttta tttcaagtca tttaaatgcc   1200
caaataggtg tttcaaatct cagatagaaa tgttcaaaag taaaaaaggt ccctatcata   1260
acataattga tatgtaagtg agttggaaaa agataagtac gtgtgagaga gatcggggat   1320
caaattctgg tgtaataatg tatgtatttc agtcataaaa attggtagca gtagttgggg   1380
ctctgtatat ataccggtaa ggatgggatg gtagtagaat aattcttttt ttgtttttag   1440
ttttttctgg tccaaaattt caaatttgga tcccttactt gtaccaacta atattaatga   1500
gtgttgaggg tagtagaggt gcaactttac cataatccct ctgtttcagg ttataagacg   1560
ttttgacttt aaatttgacc aagtttatgc gcaaatatag taatatttat aatactatat   1620
tagtttcatt aaataaataa ttgaatatat tttcataata aatttgtgtt gagttcaaaa   1680
tattattaat tttttctaca aacttggtca aacttgaagc agtttgactt tgaccaaagt   1740
caaaacgtct tataacttga aacggatgga ttactttttt tgtggggaca agtttacaat   1800
gtttaataaa gcacaatcca tcttaatgtt ttcaagctga atattgtaaa attcatggat   1860
aaaccagctt ctaaatgttt aaccgggaaa atgtcgaacg acaaattaat atttttaagt   1920
gatggggagt attaattaag gagtgacaac tcaactttca atatcgtact aaactgtggg   1980
atttattttc taaaatttta taccctgcca attcacgtgt tgtagatctt ttttttcac    2040
taaccgacac caggtatatc aattttattg aatatagcag caaaaagaat gtgttgtact   2100
tgtaaacaaa aagcaaactg tacataaaaa aaaatgcact cctatataat taagctcata   2160
aagatgcttt gcttcgtgag ggcccaagtt ttgatgacct tttgcttgat ctcgaaatta   2220
aaatttaagt actgttaagg gagttcacac caccatcaat tttcagcctg aagaaacagt   2280
taaacaacga ccccgatgac cagtctactg ctctccacat actagctgca ttattgatca   2340
caaaacaaaa caaaacgaaa taaaaatcag cagcgagagt gtgcagagag agacaaaggt   2400
gatctggcgt ggatatctcc ccatccatcc tcacccgcgc tgcccatcac tcgccgccgc   2460
atactccatc atgtggagag aggaagacga ggaccacagc cagagcccgg gtcgagatgc   2520
caccacggcc acaacccacg agcccggcgc gacaccaccg cgcgcgcgtg agccagccac   2580
aaacgcccgc ggataggcgc gcgcacgccg gccaatccta ccacatcccc ggcctccgcg   2640
gctcgcgagc gccgctgcca tccgatccgc tgagttttgg ctatttatac gtaccgcggg   2700
agcctgtgtg cagagcagtg catctcaaga agtactcgag caaagaagga gagagcttgg   2760
tgagctgcag ccatggtaga tctgagggta aatttctagt ttttctcctt cattttcttg   2820
gttaggaccc ttttctcttt ttatttttt gagctttgat ctttctttaa actgatctat    2880
tttttaattg attggttatg gtgtaaatat tacatagctt taactgataa tctgattact   2940
ttatttcgtg tgtctatgat gatgatgata gttacagaac cgacgaacta gtgcgcaggc   3000
ggttgtgcag gcgatgcagt gccaggtggg ggtgaggggc aggacggccg tcccggcgag   3060
gcagcccgcg ggcagggtgt ggggcgtcag gagggccgcc cgcgccacct ccgggttcaa   3120
ggtgctggcg ctcggcccgg agaccaccgg ggtcatccag aggatgcagc agctgctcga   3180
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catggacacc acgcccttca ccgacaagat catcgccgag tacatctggg ttggaggatc   3240

tggaattgac ctcagaagca aatcaaggac gatttcgaag ccagtggagg acccgtcaga   3300

gctgccgaaa tggaactacg acggatcgag cacggggcag gctcctgggg aagacagtga   3360

agtcatccta tacccacagg ccatattcaa ggacccattc cgaggaggca acaacatact   3420

ggttatctgt gacacctaca caccacaggg ggaacccatc cctactaaca aacgccacat   3480

ggctgcacaa atcttcagtg accccaaggt cacttcacaa gtgccatggt tcggaatcga   3540

acaggagtac actctgatgc agagggatgt gaactggcct cttggctggc ctgttggagg   3600

gtaccctggc ccccagggtc catactactg cgccgtagga tcagacaagt catttggccg   3660

tgacatatca gatgctcact acaaggcgtg cctttacgct ggaattgaaa tcagtggaac   3720

aaacggggag gtcatgcctg gtcagtggga gtaccaggtt ggacccagcg ttggtattga   3780

tgcaggagac cacatatggg cttccagata cattctcgag agaatcacgg agcaagctgg   3840

tgtggtgctc acccttgacc caaaaccaat ccagggtgac tggaacggag ctggctgcca   3900

cacaaactac agcacattga gcatgcgcga ggatggaggt ttcgacgtga tcaagaaggc   3960

aatcctgaac ctttcacttc gccatgactt gcacatagcc gcatatggtg aaggaaacga   4020

gcggaggttg acagggctac acgagacagc tagcatatca gacttctcat ggggtgtggc   4080

gaaccgtggc tgctctattc gtgtggggcg agacaccgag gcgaagggca aaggatacct   4140

ggaggaccgt cgcccggcct ccaacatgga cccgtacacc gtgacggcgc tgctggccga   4200

gaccacgatc ctgtgggagc cgaccctcga ggcggaggcc ctcgctgcca agaagctggc   4260

gctgaaggta tga                                                      4273
```

<210> SEQ ID NO 43
<211> LENGTH: 436
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Translation product of SEQ ID NO: 42 DNA

<400> SEQUENCE: 43

```
Met Val Asp Leu Arg Asn Arg Arg Thr Ser Ala Gln Ala Val Val Gln
1               5                   10                  15

Ala Met Gln Cys Gln Val Gly Val Arg Gly Arg Thr Ala Val Pro Ala
            20                  25                  30

Arg Gln Pro Ala Gly Arg Val Trp Gly Val Arg Arg Ala Ala Arg Ala
        35                  40                  45

Thr Ser Gly Phe Lys Val Leu Ala Leu Gly Pro Glu Thr Thr Gly Val
    50                  55                  60

Ile Gln Arg Met Gln Gln Leu Leu Asp Met Asp Thr Thr Pro Phe Thr
65                  70                  75                  80

Asp Lys Ile Ile Ala Glu Tyr Ile Trp Val Gly Gly Ser Gly Ile Asp
                85                  90                  95

Leu Arg Ser Lys Ser Arg Thr Ile Ser Lys Pro Val Glu Asp Pro Ser
            100                 105                 110

Glu Leu Pro Lys Trp Asn Tyr Asp Gly Ser Ser Thr Gly Gln Ala Pro
        115                 120                 125

Gly Glu Asp Ser Glu Val Ile Leu Tyr Pro Gln Ala Ile Phe Lys Asp
    130                 135                 140

Pro Phe Arg Gly Gly Asn Asn Ile Leu Val Ile Cys Asp Thr Tyr Thr
```

-continued

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145                 150                 155                 160

Pro Gln Gly Glu Pro Ile Pro Thr Asn Lys Arg His Met Ala Ala Gln
                165                 170                 175

Ile Phe Ser Asp Pro Lys Val Thr Ser Gln Val Pro Trp Phe Gly Ile
            180                 185                 190

Glu Gln Glu Tyr Thr Leu Met Gln Arg Asp Val Asn Trp Pro Leu Gly
        195                 200                 205

Trp Pro Val Gly Gly Tyr Pro Gly Pro Gln Gly Pro Tyr Tyr Cys Ala
    210                 215                 220

Val Gly Ser Asp Lys Ser Phe Gly Arg Asp Ile Ser Asp Ala His Tyr
225                 230                 235                 240

Lys Ala Cys Leu Tyr Ala Gly Ile Glu Ile Ser Gly Thr Asn Gly Glu
                245                 250                 255

Val Met Pro Gly Gln Trp Glu Tyr Gln Val Gly Pro Ser Val Gly Ile
            260                 265                 270

Asp Ala Gly Asp His Ile Trp Ala Ser Arg Tyr Ile Leu Glu Arg Ile
        275                 280                 285

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

Gly Asp Trp Asn Gly Ala Gly Cys His Thr Asn Tyr Ser Thr Leu Ser
305                 310                 315                 320

Met Arg Glu Asp Gly Gly Phe Asp Val Ile Lys Lys Ala Ile Leu Asn
                325                 330                 335

Leu Ser Leu Arg His Asp Leu His Ile Ala Ala Tyr Gly Glu Gly Asn
            340                 345                 350

Glu Arg Arg Leu Thr Gly Leu His Glu Thr Ala Ser Ile Ser Asp Phe
        355                 360                 365

Ser Trp Gly Val Ala Asn Arg Gly Cys Ser Ile Arg Val Gly Arg Asp
    370                 375                 380

Thr Glu Ala Lys Gly Lys Gly Tyr Leu Glu Asp Arg Arg Pro Ala Ser
385                 390                 395                 400

Asn Met Asp Pro Tyr Thr Val Thr Ala Leu Leu Ala Glu Thr Thr Ile
                405                 410                 415

Leu Trp Glu Pro Thr Leu Glu Ala Glu Ala Leu Ala Ala Lys Lys Leu
            420                 425                 430

Ala Leu Lys Val
        435


<210> SEQ ID NO 44
<211> LENGTH: 1992
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 44

ctgcagtgca gcgtgacccg gtcgtgcccc tctctagaga taatgagcat tgcatgtcta     60

agttataaaa aattaccaca tattttttt gtcacacttg tttgaagtgc agtttatcta    120

tctttataca tatatttaaa ctttactcta cgaataatat aatctatagt actacaataa    180

tatcagtgtt ttagagaatc atataaatga acagttagac atggtctaaa ggacaattga    240

gtattttgac aacaggactc tacagtttta tctttttagt gtgcatgtgt tctccttttt    300

ttttgcaaat agcttcacct atataatact tcatccattt tattagtaca tccatttagg    360
```

-continued

```
gtttagggtt aatggttttt atagactaat ttttttagta catctatttt attctatttt    420
agcctctaaa ttaagaaaac taaaactcta ttttagtttt tttatttaat aatttagata    480
taaaatagaa taaaataaag tgactaaaaa ttaaacaaat acccttttaag aaattaaaaa    540
aactaaggaa acatttttct tgtttcgagt agataatgcc agcctgttaa acgccgtcga    600
cgagtctaac ggacaccaac cagcgaacca gcagcgtcgc gtcgggccaa gcgaagcaga    660
cggcacggca tctctgtcgc tgcctctgga cccctctcga gagttccgct ccaccgttgg    720
acttgctccg ctgtcggcat ccagaaattg cgtggcggag cggcagacgt gagccggcac    780
ggcaggcggc ctcctcctcc tctcacggca cggcagctac gggggattcc tttcccaccg    840
ctccttcgct ttcccttcct cgcccgccgt aataaataga cacccccctcc acaccctctt    900
tccccaacct cgtgttgttc ggagcgcaca cacacacaac cagatctccc ccaaatccac    960
ccgtcggcac ctccgcttca aggtacgccg ctcgtcctcc cccccccccc ctctctacct   1020
tctctagatc ggcgttccgg tccatggtta gggcccggta gttctacttc tgttcatgtt   1080
tgtgttagat ccgtgtttgt gttagatccg tgctgctagc gttcgtacac ggatgcgacc   1140
tgtacgtcag acacgttctg attgctaact tgccagtgtt tctctttggg gaatcctggg   1200
atggctctag ccgttccgca gacgggatcg atttcatgat tttttttgtt tcgttgcata   1260
gggtttggtt tgccctttc ctttatttca atatatgccg tgcacttgtt tgtcgggtca   1320
tcttttcatg ctttttttg tcttggttgt gatgatgtgg tctggttggg cggtcgttct   1380
agatcggagt agaattctgt ttcaaactac ctggtggatt tattaatttt ggatctgtat   1440
gtgtgtgcca tacatattca tagttacgaa ttgaagatga tggatggaaa tatcgatcta   1500
ggataggtat acatgttgat gcgggtttta ctgatgcata tacagagatg ctttttgttc   1560
gcttggttgt gatgatgtgg tgtggttggg cggtcgttca ttcgttctag atcggagtag   1620
aatactgttt caaactacct ggtgtattta ttaattttgg aactgtatgt gtgtgtcata   1680
catcttcata gttacgagtt taagatggat ggaaatatcg atctaggata ggtatacatg   1740
ttgatgtggg ttttactgat gcatatacat gatggcatat gcagcatcta ttcatatgct   1800
ctaaccttga gtacctatct attataataa acaagtatgt tttataatta ttttgatctt   1860
gatatacttg gatgatggca tatgcagcag ctatatgtgg atttttttag ccctgccttc   1920
atacgctatt tatttgcttg gtactgtttc ttttgtcgat gctcaccctg ttgtttggtg   1980
ttacttctgc ag                                                       1992
```

<210> SEQ ID NO 45
<211> LENGTH: 1248
<212> TYPE: DNA
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 45

```
atggcatccg cccccgcctc cgcctccgcg gccctctcca ccgccgcccc cgccgacaac     60
ggggccgcca agcccacgga gcagcggccg gtacaggtgg ctaagcgatt ggagaagttc    120
aaaacaacaa ttttcacaca gatgagcatg ctcgcagtga agcatggagc aataaacctt    180
ggacaggggt ttcccaattt tgatggccct gactttgtca aagatgctgc tattgaggct    240
atcaaagctg gaaagaatca gtatgcaaga ggatatggtg tgcctgaatt gaactcagct    300
gttgctgaga gatttctcaa ggacagtgga ttgcacatcg atcctgataa ggaagttact    360
```

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```
gttacatctg ggtgcacaga agcaatagct gcaacgatat tgggtctgat caaccctggg    420

gatgaagtca tactgtttgc tccattctat gattcttatg aggctacact gtccatggct    480

ggtgcgaatg tcaaagccat tacactccgc cctccggact ttgcagtccc tcttgaagag    540

ctaaaggctg cagtctcgaa gaataccaga gcaataatga ttaatacacc tcacaaccct    600

accgggaaaa tgttcacaag ggaggaactt gagttcattg ctgatctctg caaggaaaat    660

gacgtgttgc tctttgccga tgaggtctac gacaagctgg cgtttgaggc ggatcacata    720

tcaatggctt ctattcctgg catgtatgag aggaccgtca ctatgaactc cctggggaag    780

acgttctcct tgaccggatg gaagatcggc tgggcgatag caccaccgca cctgacatgg    840

ggcgtaaggc aggcacactc cttcctcaca ttcgccacct ccacgccgat gcaatcagca    900

gcggcggcgg ccctgagagc accggacagc tactttgagg agctgaagag ggactacggc    960

gcaaagaaag cgctgctggt ggacgggctc aaggcggcgg gcttcatcgt ctacccttcg   1020

agcggaacct acttcatcat ggtcgaccac accccgttcg ggttcgacaa cgacgtcgag   1080

ttctgcgagt acttgatccg cgaggtcggc gtcgtggcca tcccgccaag cgtgttctac   1140

ctgaacccgg aggacgggaa gaacctggtg aggttcacct tctgcaagga cgacgacacg   1200

ctaagggcgg cggtggacag gatgaaggcc aagctcagga agaaatga                1248
```

<210> SEQ ID NO 46
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 46

```
Met Ala Ser Ala Pro Ala Ser Ala Ser Ala Ala Leu Ser Thr Ala Ala
1               5                   10                  15

Pro Ala Asp Asn Gly Ala Ala Lys Pro Thr Glu Gln Arg Pro Val Gln
            20                  25                  30

Val Ala Lys Arg Leu Glu Lys Phe Lys Thr Thr Ile Phe Thr Gln Met
        35                  40                  45

Ser Met Leu Ala Val Lys His Gly Ala Ile Asn Leu Gly Gln Gly Phe
    50                  55                  60

Pro Asn Phe Asp Gly Pro Asp Phe Val Lys Asp Ala Ala Ile Glu Ala
65                  70                  75                  80

Ile Lys Ala Gly Lys Asn Gln Tyr Ala Arg Gly Tyr Gly Val Pro Glu
                85                  90                  95

Leu Asn Ser Ala Val Ala Glu Arg Phe Leu Lys Asp Ser Gly Leu His
            100                 105                 110

Ile Asp Pro Asp Lys Glu Val Thr Val Thr Ser Gly Cys Thr Glu Ala
        115                 120                 125

Ile Ala Ala Thr Ile Leu Gly Leu Ile Asn Pro Gly Asp Glu Val Ile
    130                 135                 140

Leu Phe Ala Pro Phe Tyr Asp Ser Tyr Glu Ala Thr Leu Ser Met Ala
145                 150                 155                 160

Gly Ala Asn Val Lys Ala Ile Thr Leu Arg Pro Pro Asp Phe Ala Val
                165                 170                 175

Pro Leu Glu Glu Leu Lys Ala Ala Val Ser Lys Asn Thr Arg Ala Ile
            180                 185                 190

Met Ile Asn Thr Pro His Asn Pro Thr Gly Lys Met Phe Thr Arg Glu
        195                 200                 205
```

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```
Glu Leu Glu Phe Ile Ala Asp Leu Cys Lys Glu Asn Asp Val Leu Leu
    210                 215                 220

Phe Ala Asp Glu Val Tyr Asp Lys Leu Ala Phe Glu Ala Asp His Ile
225                 230                 235                 240

Ser Met Ala Ser Ile Pro Gly Met Tyr Glu Arg Thr Val Thr Met Asn
                245                 250                 255

Ser Leu Gly Lys Thr Phe Ser Leu Thr Gly Trp Lys Ile Gly Trp Ala
            260                 265                 270

Ile Ala Pro Pro His Leu Thr Trp Gly Val Arg Gln Ala His Ser Phe
        275                 280                 285

Leu Thr Phe Ala Thr Ser Thr Pro Met Gln Ser Ala Ala Ala Ala Ala
    290                 295                 300

Leu Arg Ala Pro Asp Ser Tyr Phe Glu Glu Leu Lys Arg Asp Tyr Gly
305                 310                 315                 320

Ala Lys Lys Ala Leu Leu Val Asp Gly Leu Lys Ala Ala Gly Phe Ile
                325                 330                 335

Val Tyr Pro Ser Ser Gly Thr Tyr Phe Ile Met Val Asp His Thr Pro
            340                 345                 350

Phe Gly Phe Asp Asn Asp Val Glu Phe Cys Glu Tyr Leu Ile Arg Glu
        355                 360                 365

Val Gly Val Val Ala Ile Pro Pro Ser Val Phe Tyr Leu Asn Pro Glu
    370                 375                 380

Asp Gly Lys Asn Leu Val Arg Phe Thr Phe Cys Lys Asp Asp Asp Thr
385                 390                 395                 400

Leu Arg Ala Ala Val Asp Arg Met Lys Ala Lys Leu Arg Lys Lys
                405                 410                 415
```

<210> SEQ ID NO 47
<211> LENGTH: 2735
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic expression cassette

<400> SEQUENCE: 47

```
gtttgaatcc tccttaaagt ttttctctgg agaaactgta gtaattttac tttgttgtgt     60

tcccttcatc ttttgaatta atggcatttg ttttaatact aatctgcttc tgaaacttgt    120

aatgtatgta tatcagtttc ttataattta tccaagtaat atcttccatt ctctatgcaa    180

ttgcctgcat aagctcgaca aaagagtaca tcaacccctc ctcctctgga ctactctagc    240

taaacttgaa tttcccctta agattatgaa attgatatat ccttaacaaa cgactccttc    300

tgttggaaaa tgtagtactt gtctttcttc ttttgggtat atatagttta tatacaccat    360

actatgtaca acatccaagt agagtgaaat ggatacatgt acaagactta tttgattgat    420

tgatgacttg agttgcctta ggagtaacaa attcttaggt caataaatcg ttgatttgaa    480

attaatctct ctgtcttaga cagataggaa ttatgacttc caatggtcca gaaagcaaag    540

ttcgcactga gggtatactt ggaattgaga cttgcacagg tccagaaacc aaagttccca    600

tcgagctcta aaatcacatc tttggaatga aattcaatta gagataagtt gcttcatagc    660

ataggtaaaa tggaagatgt gaagtaacct gcaataatca gtgaaatgac attaatacac    720

taaatacttc atatgtaatt atcctttcca ggttaacaat actctataaa gtaagaatta    780
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```
tcagaaatgg gctcatcaaa cttttgtact atgtatttca tataaggaag tataactata    840
cataagtgta tacacaactt tattcctatt ttgtaaaggt ggagagactg ttttcgatgg    900
atctaaagca atatgtctat aaaatgcatt gatataataa ttatctgaga aaatccagaa    960
ttggcgttgg attatttcag ccaaatagaa gtttgtacca tacttgttga ttccttctaa   1020
gttaaggtga agtatcattc ataaacagtt ttccccaaag tactactcac caagtttccc   1080
tttgtagaat taacagttca aatatatggc gcagaaatta ctctatgccc aaaaccaaac   1140
gagaaagaaa caaaatacag gggttgcaga ctttattttc gtgttagggt gtgtttttc   1200
atgtaattaa tcaaaaaata ttatgacaaa aacatttata catattttta ctcaacactc   1260
tgggtatcag ggtgggttgt gttcgacaat caatatggaa aggaagtatt ttccttattt   1320
ttttagttaa tattttcagt tataccaaac ataccttgtg atattatttt taaaaatgaa   1380
aaactcgtca gaaagaaaaa gcaaaagcaa caaaaaaatt gcaagtattt tttaaaaaag   1440
aaaaaaaaaa catatcttgt ttgtcagtat gggaagtttg agataaggac gagtgagggg   1500
ttaaaattca gtggccattg attttgtaat gccaagaacc acaaaatcca atggttacca   1560
ttcctgtaag atgaggtttg ctaactcttt ttgtccgtta gataggaagc cttatcacta   1620
tatatacaag gcgtcctaat aacctcttag taaccaatta tttcagcacc atgtctctgc   1680
tctcagatct cgttaacctc aacctcaccg atgccaccgg gaaaatcatc gccgaataca   1740
tatggatcgg tggatctgga atggatatca gaagcaaagc caggacacta ccaggaccag   1800
tgactgatcc atcaaagctt cccaagtgga actacgacgg atccagcacc ggtcaggctg   1860
ctggagaaga cagtgaagtc attctatacc ctcaggcaat attcaaggat cccttcagga   1920
aaggcaacaa catcctggtg atgtgtgatg cttacacacc agctggtgat cctattccaa   1980
ccaacaagag gcacaacgct gctaagatct tcagccaccc cgacgttgcc aaggaggagc   2040
cttggtatgg gattgagcaa gaatacactt tgatgcaaaa ggatgtgaac tggccaattg   2100
gttggcctgt tggtggctac cctggccctc agggacctta ctactgtggt gtgggagctg   2160
acaaagccat tggtcgtgac attgtggatg ctcactacaa ggcctgtctt tacgccggta   2220
ttggtatttc tggtatcaat ggagaagtca tgccaggcca gtgggagttc caagtcggcc   2280
ctgttgaggg tattagttct ggtgatcaag tctgggttgc tcgatacctt ctcgagagga   2340
tcactgagat ctctggtgta attgtcagct tcgacccgaa accagtcccg ggtgactgga   2400
atggagctgg agctcactgc aactacagca ctaagacaat gagaaacgat ggaggattag   2460
aagtgatcaa gaaagcgata gggaagcttc agctgaaaca caaagaacac attgctgctt   2520
acggtgaagg aaacgagcgt cgtctcactg gaaagcacga aaccgcagac atcaacacat   2580
tctcttgggg agtcgcgaac cgtggagcgt cagtgagagt gggacgtgac acagagaagg   2640
aaggtaaagg gtacttcgaa gacagaaggc cagcttctaa catggatcct tacgttgtca   2700
cctccatgat cgctgagacg accatactcg gttga                              2735
```

<210> SEQ ID NO 48
<211> LENGTH: 1371
<212> TYPE: DNA
<213> ORGANISM: Citrus reticulata

<400> SEQUENCE: 48

```
atgcttaagc cgtccgcctt cgggtcttct ttttcttcct cagctctgct ttcgttttcg     60
```

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aagcatttgc atacaataag cattactgat tctgtcaaca ccagaagaag aggaatcagt    120
accgcttgcc ctaggtaccc ttctctcatg gcgagcttgt ccaccgtttc caccaatcaa    180
agcgacacca tccagaagac caatcttcag cctcaacagg ttgctaagtg cttggagaag    240
tttaaaacta caatctttac acaaatgagt atgcttgcca tcaaacatgg agctataaat    300
cttggtcaag gctttcccaa ctttgatggc ccagattttg ttaaagatgc agcgattcaa    360
gccataaggg atgggaagaa tcaatatgct cgtggacatg gggttccaga gttcaactct    420
gccattgctt cccggtttaa gaaagattct gggctcgagg ttgaccctga aaaggaagtt    480
actgttacct ctgggtgcac cgaagccatt gctgcaacca tcttaggttt gattaatcct    540
ggagatgagg tgatcctttt tgcacctttc tatgattcct atgaagctac tctctccatg    600
gctggtgcta aaattaaatg catcacattg cgccctccag aatttgccat ccccattgaa    660
gagctcaagt ctacaatctc aaaaaatact cgtgcaattc ttatgaacac tccacataac    720
cccactggaa agatgttcac tagggaggaa cttaatgtta ttgcatctct ttgcattgag    780
aatgatgtgt tggtttttag tgatgaggtc tatgataagt tggcttttga aatggatcac    840
atttccatag cctctcttcc tggaatgtat gagcgtactg taaccatgaa ttccttaggg    900
aagacattct ctttaacagg gtggaagatc gggtgggcaa tagctccacc gcaccttaca    960
tgggggtgc ggcaggcaca ctcttttctc acgtttgcca catccactcc aatgcagtgg   1020
gcagctacag cagcccttag agctccggag acgtactatg aggagctaaa gagagattac   1080
tcggcaaaga aggcaatttt ggtggaggga ttgaatgctg ttggtttcaa ggtattccca   1140
tctagtggga catactttgt ggttgtagat cacaccccat ttgggcacga aactgatatt   1200
gcattttgtg aatatctgat caaggaagtt ggggttgtgg caattccgac cagcgtattt   1260
tacttgaatc cagaggatgg aaagaatttg gtgagattta ccttctgcaa agatgaagga   1320
actttgaggt ctgcagttga caggatgaag gagaagctga ggagaaaatg a            1371
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<210> SEQ ID NO 49
<211> LENGTH: 456
<212> TYPE: PRT
<213> ORGANISM: Citrus reticulata

<400> SEQUENCE: 49

```
Met Leu Lys Pro Ser Ala Phe Gly Ser Ser Phe Ser Ser Ser Ala Leu
1               5                   10                  15

Leu Ser Phe Ser Lys His Leu His Thr Ile Ser Ile Thr Asp Ser Val
            20                  25                  30

Asn Thr Arg Arg Arg Gly Ile Ser Thr Ala Cys Pro Arg Tyr Pro Ser
        35                  40                  45

Leu Met Ala Ser Leu Ser Thr Val Ser Thr Asn Gln Ser Asp Thr Ile
    50                  55                  60

Gln Lys Thr Asn Leu Gln Pro Gln Gln Val Ala Lys Cys Leu Glu Lys
65                  70                  75                  80

Phe Lys Thr Thr Ile Phe Thr Gln Met Ser Met Leu Ala Ile Lys His
                85                  90                  95

Gly Ala Ile Asn Leu Gly Gln Gly Phe Pro Asn Phe Asp Gly Pro Asp
            100                 105                 110

Phe Val Lys Asp Ala Ala Ile Gln Ala Ile Arg Asp Gly Lys Asn Gln
        115                 120                 125
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Tyr Ala Arg Gly His Gly Val Pro Glu Phe Asn Ser Ala Ile Ala Ser
    130                 135                 140

Arg Phe Lys Lys Asp Ser Gly Leu Glu Val Asp Pro Glu Lys Glu Val
145                 150                 155                 160

Thr Val Thr Ser Gly Cys Thr Glu Ala Ile Ala Ala Thr Ile Leu Gly
                165                 170                 175

Leu Ile Asn Pro Gly Asp Glu Val Ile Leu Phe Ala Pro Phe Tyr Asp
            180                 185                 190

Ser Tyr Glu Ala Thr Leu Ser Met Ala Gly Ala Lys Ile Lys Cys Ile
        195                 200                 205

Thr Leu Arg Pro Pro Glu Phe Ala Ile Pro Ile Glu Glu Leu Lys Ser
    210                 215                 220

Thr Ile Ser Lys Asn Thr Arg Ala Ile Leu Met Asn Thr Pro His Asn
225                 230                 235                 240

Pro Thr Gly Lys Met Phe Thr Arg Glu Glu Leu Asn Val Ile Ala Ser
                245                 250                 255

Leu Cys Ile Glu Asn Asp Val Leu Val Phe Ser Asp Glu Val Tyr Asp
            260                 265                 270

Lys Leu Ala Phe Glu Met Asp His Ile Ser Ile Ala Ser Leu Pro Gly
        275                 280                 285

Met Tyr Glu Arg Thr Val Thr Met Asn Ser Leu Gly Lys Thr Phe Ser
    290                 295                 300

Leu Thr Gly Trp Lys Ile Gly Trp Ala Ile Ala Pro Pro His Leu Thr
305                 310                 315                 320

Trp Gly Val Arg Gln Ala His Ser Phe Leu Thr Phe Ala Thr Ser Thr
                325                 330                 335

Pro Met Gln Trp Ala Ala Thr Ala Ala Leu Arg Ala Pro Glu Thr Tyr
            340                 345                 350

Tyr Glu Glu Leu Lys Arg Asp Tyr Ser Ala Lys Lys Ala Ile Leu Val
        355                 360                 365

Glu Gly Leu Asn Ala Val Gly Phe Lys Val Phe Pro Ser Ser Gly Thr
    370                 375                 380

Tyr Phe Val Val Val Asp His Thr Pro Phe Gly His Glu Thr Asp Ile
385                 390                 395                 400

Ala Phe Cys Glu Tyr Leu Ile Lys Glu Val Gly Val Val Ala Ile Pro
                405                 410                 415

Thr Ser Val Phe Tyr Leu Asn Pro Glu Asp Gly Lys Asn Leu Val Arg
            420                 425                 430

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

Met Lys Glu Lys Leu Arg Arg Lys
    450                 455


<210> SEQ ID NO 50
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer sequence

<400> SEQUENCE: 50

ggccacatgt ccgttgctaa gtgcttggag aagttta                              37
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-continued

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<210> SEQ ID NO 51
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer sequence

<400> SEQUENCE: 51

cgggcacgtg tcattttctc ctcagcttct ccttcatcct                          40
```

1. A transgenic plant comprising a GPT transgene and a GS transgene, wherein each of said GPT transgene and said GS transgene is operably linked to a plant promoter.

2. (canceled)

3. The transgenic plant of claim **1**, wherein each plant promoter is heterologous to the plant.

4. The transgenic plant of claim **1**, wherein the plant promoter operably linked to the GPT transgene is heterologous to the species from which the GPT transgene is derived, and wherein the plant promoter operably linked to the GS transgene is heterologous to the species from which the GS transgene is derived.

5. The transgenic plant of claim **4**, wherein the GPT transgene encodes a polypeptide having an amino acid sequence selected from the group consisting of (a) SEQ ID NO: 2; SEQ ID NO: 9; SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO 24, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 46 and SEQ ID NO: 49, and (b) an amino acid sequence that is at least 75% identical to any one of SEQ ID NO: 2; SEQ ID NO: 9; SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO 24, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 46 and SEQ ID NO: 49 and has GPT activity.

6. The transgenic plant of claim **4**, wherein the GS transgene encodes a polypeptide having an amino acid sequence selected form the group consisting of (a) SEQ ID NO: 4, SEQ ID NO: 7 from residue 11, and SEQ ID NO: 41, and (b) an amino acid sequence that is at least 75% identical to SEQ ID NO: 4, SEQ ID NO: 7 or SEQ ID NO: 41.

7. The transgenic plant according to claim **1**, wherein the GPT and GS transgenes are incorporated into the genome of the plant.

8. The transgenic plant according to claim **1**, wherein the GPT and/or the GS transgenes are incorporated into the plastome of the plant.

9-15. (canceled)

16. The transgenic plant according to claim **1**, which is a plant of the phylum *Tracheophyta*.

17. The transgenic plant of claim **16**, wherein the transgenic plant is selected from the group consisting of fruits including apples, avocado, banana, blackberry, blueberry, cherries, cranberries, cantaloupes, grapefruit, lemons, limes, nectarines, oranges, peaches, pineapples, pears, plums, tangelos, tangerines, papaya, mango, strawberry, raspberry, grape, kiwi fruit, olives, and coconuts; vegetables including brussel sprouts, radish, mung beans, carrots, sugarbeet, yams, horseradish, flax, sesame, turnip, sweet potato, cabbage, canola, cucumbers, eggplant, lettuce, onion, okra, parsnips, pumpkins and spinach; flowering plants including lily, carnation, chrysanthemum, petunia, geranium, violet, gladioli, orchid, and lilac; trees including oil palm; ornamental plants; canola; lupins; and cotton.

18. The transgenic plant of claim **16**, wherein the transgenic plant is selected from the group consisting of plants of the families Poaceae, *Gossypium*, Fabaceae, Brassicaceae, Rutaceae, Rubiaceae, Cucurbitaceae, Rosaceae, Asteraceae, Amaranthaceae and Brassicaceae.

19. The transgenic plant of claim **16**, wherein the transgenic plant is selected from the group consisting of plants of the genera *Avena, Hordeum, Oryza, Panicum, Phleum, Saccharum, Secale, Sorghum, Triticum, Zea, Pennisetum, Lycopersicon, Capiscum, Fagopyrum, Triticosecale, Chenopodium, Digitaria, Manihot, Ipomoea, Olea, Daucus, Pastinaca, Raphanus, Dioscorea, Armoracia, Elaeis, Linum, Carthamus, Sesamum, Vitis*, and *Solanum*.

20-22. (canceled)

23. A method of producing a plant having increased nitrogen use efficiency relative to an analogous wild type or untransformed plant, comprising:

(a) introducing a GPT transgene into the plant;

(b) introducing a GS transgene into the plant or a progeny of the plant;

(c) expressing the GPT transgene and the GS transgene in the plant or the progeny of the plant; and,

(d) selecting a plant having an increased nitrogen use efficiency relative to a plant of the same species that does not comprise a GPT transgene or a GS transgene.

24-29. (canceled)

30. A transplastomic plant or cell line carrying a GPT transgene and a GS transgene expression cassette, wherein said expression cassette is flanked by sequences from the plant or plant cell's plastome.

31-35. (canceled)

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