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(57) Abstract

A method of producing a plant with switchable starch-synthesising ability comprises stably incorporating into the genome of a recipient plant at least one target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and under the control of a gene switch. A plant with switchable starch-synthesising ability may have switchable starch yield, and/or switchable starch quality. Starch or glycogen biosynthetic enzymes include soluble starch synthase, branching enzyme, glycogen synthase, ADP-glucose pyrophosphorylase, self-glucosylating protein, glycogenin and amylogenin. DNA constructs for use in this method are described, as well as plants transformed with said DNA constructs, the seeds and progeny of such plants, and hybrids whose pedigree includes such plants.

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NOVEL PLANTS AND PROCESSES FOR OBTAINING THEM

This invention relates to novel plants having an altered ability to produce starch, including an improved ability to produce structurally-altered starch or starch of altered quality. The invention further relates to processes for obtaining such plants.

Agriculture uses many crop plants for the production of food for human or animal consumption, for commercial processes yielding products for human consumption, for the development of industrial products and for other purposes. Traditionally, the improvement of crop plant species involves the introduction of desired traits by genetic crosses. These breeding techniques are highly successful, and seed producers sell the resulting seed to the farmer. The farmer plants this seed and harvests the crop, be it the whole plant, its seed or its fruit. The crop is then used for the various applications mentioned above.

Starch is an important end-product of carbon fixation during photosynthesis in leaves and is an important storage product in seeds and fruits. In economic terms, the starch produced by the edible portions of three grain crops, wheat, rice and maize, provide approximately two-thirds of the world's food calculated as calories.

Many types of crops produce and store starch, including cereals, fruit, roots and oilseeds.

Starch (amylose and amylopectin) is synthesised in the plastid compartment (the chloroplast in photosynthetic cells or the amyloplast in non-photosynthetic cells). This starch is used to produce a wide range of food products (for human and animal consumption) and industrial products (such as glue). Several crop varieties are known which produce different types of starch. The type or quality of starch makes it suitable for certain purposes, including particular methods of processing or particular end-uses. For example, US Patent Serial Numbers 4789557, 4790997, 4774328, 4770710, 4798735, 4767849, 4801470, 4789738, 4792458 and 5009911 describe naturally-occurring maize mutants producing starches of differing fine structure suitable for use in various food products. These mutants include the dull, waxy, amylose extender, shrunken, sugary and floury mutants. Although known mutants produce altered starch, some of these lines are not suitable for crop breeding and/or for the farmers' purposes. For example, they often give relatively poor yields.

Improved crops may be produced by genetic manipulation of plants known to possess other favourable characteristics. By manipulating the expression of one or more starch-synthesising enzyme genes, it is possible to alter the amount and/or type of starch produced in a plant. One or more enzyme gene constructs, which may be of plant, fungal, bacterial or animal origin, are incorporated into the plant genome by sexual crossing or by transformation. The enzyme gene may

be an additional copy of the wild-type gene or may encode a modified or allelic or alternative enzyme with improved properties. Incorporation of the enzyme gene construct(s) may have varying effects depending on the amount and type of enzyme gene(s) introduced (in a sense or antisense orientation). It may increase the plant's capacity to produce starch, in particular by altering the temperature optimum for enzyme activity, giving increased yield. It may also result in production of starch with an altered fine structure (or quality) as the exact structure depends on the balance of the different enzymes. The following patent applications describe this concept in detail: US application number 948280 and equivalent International application number GB92/01881; US application number 930935 and equivalent European publication number EPA 368506 (published 16 May 1990); International application number GB93/01821. The disclosures of these applications are hereby incorporated by reference.

So numerous crop lines are known which produce starches of differing fine structure (that is, differing quality). These lines may be naturally-occurring mutants or may have been produced by genetic manipulation (using traditional breeding or biotechnological techniques).

It would be advantageous to be able to control the starch-producing ability of the crop according to circumstances. If the relevant enzyme genes could be switched on or off at will, the properties or quality of the crop could be directly

controlled. Thus the farmer or seed producer would have the ability to grow crops having a different type/quality of starch as desired for different purposes (eg for different methods of processing; for production of varying food sources; for seed production).

A particular problem with known lines producing structurally-altered starch is that the quantity of starch produced in the crop is relatively low because:
(i) germinability of the seed is poor (due to a lower starch content), and
(ii) the normal functioning of the starch enzymes is disrupted (so a lower yield of starch is deposited in the seed).

Several gene promoter sequences are known which are responsive to an applied exogenous chemical inducer. This enables external control of expression of the gene controlled by the inducible promoter. For example, European patent application publication number EPA 332104 (published 18th September 1989) describes chemically regulatable DNA sequences isolated from the pathogenesis-related (PR) protein gene; International patent application publication numbers WO90/08826 (published 9 August 1990) and WO93/01294 (published 21 January 1993) describe a chemically inducible gene promoter sequence isolated from a 27kd subunit of the maize glutathione-S-transferase gene (GST II-27); International patent application number GB93/00764 describes a chemically-inducible gene expression

cassette including a regulator protein (such as the Aspergillus nidulans alcR protein) and an inducible promoter (such as the A nidulans alcA promoter).

Such chemically-inducible promoter sequences may be used in "gene switches" to regulate transcription of an associated DNA sequence (or "target gene") in plants or plant tissue.

The gene switch may be a positive switch, where the inducible promoter directly controls the target gene. In the presence of the chemical inducer, the target gene is switched on and the encoded protein is expressed.

For example, the inducible GST II-27 promoter can be operatively linked to one or more target genes to give a chemically switchable construct: expression of the target gene(s) is controlled by application of an effective exogenous inducer. The gene switch construct may be inserted into a plant by transformation. The inducible GST II-27 promoter is functional in both monocotyledons and dicotyledons, and in a variety of tissues including roots, leaves, stems and reproductive tissues. Effective inducers for use with the GST II-27 promoter include N,N-diallyl-2,2-dichloroacetamide (common name: dichloramid); benzyl-2-chloro-4-(trifluoromethyl) -5-thiazole-carboxylate (common name: flurazole); naphthalene-1,8-dicarboxylic anhydride; 2-dichloromethyl-2-methyl-1,3-dioxolane and several others as described in International patent application publication numbers WO90/08826 and

WO93/01294. The contents of the said applications are incorporated herein by reference.

Alternatively, the gene switch may be a negative switch, where the inducible promoter indirectly controls the target gene via a repressor/operator system. In the presence of the chemical inducer, the target gene is switched off and the encoded protein is not expressed.

For example, negative gene switches are described in International patent application publication numbers WO90/08829, WO90/08827 and WO90/08830 (all published 9 August 1990). The contents of the said applications are incorporated herein by reference. The switch comprises a chemically-inducible promoter (A) driving expression of a repressor gene encoding a repressor protein, and a promoter (B) containing an operator sequence and driving expression of a target gene. (The operator region may be introduced into promoter(B) by biotechnological techniques). If present, the repressor protein binds to the operator sequence, preventing expression of the target gene. In the absence of inducer, promoter (A) is not active and the repressor protein is not expressed: hence the target gene is expressed. In the presence of the chemical inducer, the repressor protein prevents expression of the target gene. Promoter (A) may be GST II-27 or any other chemically-inducible promoter sequence. The repressor gene/operator sequences may be taken from the E coli lac operon.

An object of the present invention is to provide a mechanism by which the expression of specific starch-synthesising enzyme genes may be regulated at will.

According to the present invention there is provided a method of producing a plant with switchable starch-synthesising ability comprising stably incorporating into the genome of a recipient plant at least one target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and under the control of a gene switch.

A plant with switchable starch-synthesising ability may have switchable starch yield, and/or (preferably) switchable starch quality.

Crops with switchable starch yield have a chemically regulatable ability to synthesise starch in differing amounts and/or at differing rates and/or at differing temperature optima. Expression of the target gene(s) (inserted in a sense and/or an anti-sense orientation) effects a change in the activities and/or natural ratios of the enzymes or their isoforms which results in the production of differing quantities of starch.

Crops with switchable starch quality have a chemically regulatable ability to synthesise starch with an altered fine structure. Expression of the target gene(s) (inserted in a sense and/or an anti-sense orientation) effects a change in the activites and/or natural ratios of the enzymes or their isoforms which results in the production of

differing qualities of starch. For example, the fine branching structure of starch is determined by the overall activities of the various isoforms of the starch synthases and branching enzymes being expressed during starch deposition in the developing endosperm. Altering the activities and/or ratios of starch synthetase and branching enzyme and/or the source of the enzymes (eg replacing maize starch synthase with pea starch synthase) alters the fine-branching structure of the starch.

The invention further provides a DNA construct which comprises at least one target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and under the control of a gene switch.

The invention also provides plants transformed with said DNA construct, the seeds and progeny of such plants, and hybrids whose pedigree includes such plants.

Preferably, the target gene encodes one or more of the following enzymes: soluble starch synthase (SSS) (E.C. 2.4.1.21); branching enzyme (BE) (E.C. 2.4.1.18); glycogen synthase (GS) of bacterial origin (E.C. 2.4.1.21) or animal origin (E.C. 2.4.1.11); ADP-glucose pyrophosphorylase; glycogenin, amylogenin or self glucosylating protein (SGP).

The target gene is obtainable from any suitable bacterial, fungal (including yeast), plant

or animal source. The target gene may be derived from cDNA or genomic DNA (gDNA) encoding a starch or glycogen synthetic enzyme, or it may be synthesised ab initio using standard techniques.

The target gene encodes at least part of an enzyme involved in a starch or glycogen biosynthetic pathway. The target gene may encode the complete enzyme in the sense orientation so that the transcription product (mRNA) can be translated into the active enzyme. Alternatively, the target gene may encode a portion of the enzyme in the sense orientation or may encode some or all of the enzyme in the antisense orientation so that the transcribed mRNA inhibits expression of the enzyme. It is possible to insert more than one copy of the target gene into the recipient plant genome. At least one of the target genes may encode a modified allelic form of the enzyme having altered characteristics (such as increased or decreased activity, or differing interactions with other enzymes).

When the enzyme encoded by the target gene must be expressed within the plastid compartment, the protein must be transported into the amyloplast (or chloroplast) by means of a transit peptide. A suitable transit peptide-encoding sequence must therefore precede the target gene sequence.

The gene switch may be a positive or a negative switch which is responsive to a chemical inducer. The gene switch includes a gene promoter which is inducible by application of an exogenous

chemical inducer, and which is operatively linked (directly or indirectly) to control expression of the target gene within a plant genome. It gives the ability to switch the target gene(s) on or off as desired. Using a positive gene switch (inducible promoter alone), presence of a chemical inducer switches the target gene on and the crop contains starch of altered fine structure. In the absence of a chemical inducer, the target gene is inactive and starch has its "normal" structure. Using a negative gene switch (inducible promoter with repressor/operator system), the target gene is switched on in the absence of chemical inducer, giving starch of altered fine structure. The target gene is switched off in the presence of chemical inducer giving starch of "normal" structure.

The inducible promoter may be the gene promoter for the 27 kD subunit of the glutathione-S-transferase, isoform II, enzyme (GST II-27), although it is clear that additional chemically induced promoters may be used. Some of these may be of plant origin, others may be of fungal (including yeast) origin.

A genomic DNA sequence encoding the GST II-27 gene promoter (having the nucleotide sequence shown in Figure 5) was deposited on 14 June 1991 in the National Collections of Industrial and Marine Bacteria (NCIMB), 23 St Machar Drive, Aberdeen, AB2 1RY, Scotland, UK, as plasmid pGIE7 contained within Escherichia coli, strain XLI-Blue with the accession number NCIMB 40426. A cDNA sequence

(having the nucleotide sequence shown in Figure 6) encoding this GST II-27 subunit was deposited on 19 April 1991 in NCIMB as plasmid pIJ21 contained within Escherichia coli, strain XLI-Blue with the accession number NCIMB 40413.

The plasmid p35SlacI containing DNA encoding a repressor/operator system as described in International patent application publication number WO90/08829 was deposited in an E.coli, strain TG-2, host with the National Collection of Industrial and Marine Bacteria Limited, Aberdeen, United Kingdom, on 12th December 1988, under the Accession Number NCIB 40092. The plasmid pAD18 described in International patent application publication number WO90/08827, which contains the lac operon (lacZ and lacY genes under the control of the lac promoter/operator), has been deposited under the terms of the Budapest Treaty, in an Escherichia coli, strain DH5α, host, with The National Collections of Industrial and Marine Bacteria Limited, Aberdeen, United Kingdom, on 21 December 1988, under the accession Number 40096. The plasmid pPS1, a derivative of pAD18, was also deposited under the terms of the Budapest Treaty, in an Escherichia coli, strain DH5α, host, with NCIMB on 21 December 1988, under the accession Number 40097.

The target gene(s) are incorporated into the genome of the recipient plant by sexual crossing or by transformation. The method employed for transformation of the plant cells is not especially germane to this invention and any method suitable

for the target plant may be employed. Numerous transformation procedures are known from the literature such as agroinfection using Agrobacterium tumefaciens or its Ti plasmid, electroporation, microinjection of plant cells and protoplasts, microprojectile transformation and pollen tube transformation, to mention but a few. Reference may be made to the literature for full details of the known methods. The transformed cells may then be regenerated into whole transgenic plants in which the new nuclear material is stably incorporated into the genome. Both transformed monocot and dicot plants may be obtained in this way, although the latter are usually more easy to regenerate.

The above method is generally applicable to all plants producing or storing starch. The recipient plant may be: a cereal such as maize (corn), wheat, rice, sorghum or barley; a fruit-producing species such as banana, apple, tomato or pear; a root crop such as cassava, potato, yam or turnip; an oilseed crop such as rapeseed, sunflower, oil palm, coconut, linseed or groundnut; a meal crop such as soya, bean or pea; or any other suitable species. Preferably the recipient plant is of the family Gramineae and most preferably of the species Zea mays.

The method according to the invention may be used to produce a plant having a chemically regulatable ability to synthesise starch with an altered fine structure. The plant has switchable starch quality: the type of starch it produces is

responsive to a chemical inducer and so can be externally controlled. It is thus possible to generate crops at will which produce starch better adapted or targetted to the crops' end-use (such as starch for varying food products, with improved processing properties, with improved digestibility, with improved seed production characteristics, etc).

As stated previously, a particular problem with known lines producing structurally-altered starch is that the quantity of starch produced in the crop is relatively low because:
(i) germinability of the seed is poor (due to a lower starch content), and
(ii) the normal functioning of the starch enzymes is disrupted (so a lower yield of starch is deposited in the seed).
By virtue of this invention, it is possible to switch off the production of altered starch during seed production by a seed producer so that seed with normal starch and hence normal germinability is produced. The farmer then plants the normal seed (good germinability) and switches on the production of altered starch in the emerging plants. Hence problem (ii) above will still apply in the farmer's field, but as germinability is good the overall yield is high enough.

For example, the crops grown by the farmer will need the target enzyme genes switched on to give altered starch structure in the seed produced. The farmer benefits from improved starch quality ("improved" with respect to its intended end-use).

Although there is a slight decrease in the total amount of starch in the seed (ie lower yield) which lowers its germinability, this is no disadvantage to the farmer as he will not be re-growing the seed and the improved starch quality compensates for any reduced yield. However, the crops grown by seed producers for seed production will need the target enzyme genes switched off as the "improved quality" starch is not required. Instead, the normal type and amount of starch is required for optimal germination: the seed company merely needs as many normal seeds as possible to maximise production. The seed companies can then supply the "normal" seed to farmers which germinates well to give a good stand. As the crop grows, the target enzyme gene(s) may be switched on by the farmer. A preferred embodiment is to use a negative gene switch (inducible promoter with repressor/operator system), so that presence of chemical inducer is needed to switch the enzyme genes off. Hence the farmer does not need to spray his crop with chemicals.

The main advantage of the switchable quality crops described above is that plant growth and vigour is guaranteed in the seed production fields and in the farmers' fields, while allowing the farmer to produce seed with altered starch fine structure/improved starch quality at the appropriate time using the same crop variety.

Another example of a specific application of the invention is the production of switchable or inducible sweetcorn. This gives the beneficial

ability to produce a different type of corn as desired.

The shrunken2 or the brittle2 sweetcorn mutant carries a mutation in the ADPG pyrophosphorylase gene such that sucrose content is the grain increases with a corresponding decrease in starch content. Expression of the normal ADPG pyrophosphorylase gene within a sweetcorn line allows production of normal starch. If sweetcorn plants are transformed with a normal ADPG pyrophosphorylase gene under the control of a positive or negative gene switch, the crop grower has the ability to switch between production of normal starch or sweetcorn by switching the normal ADPG pyrophosphorylase gene on or off as desired. With a positive gene switch, sweetcorn is produced in the absence of inducer. With a negative gene switch, sweetcorn is produced in the presence of inducer.

Alternatively, inhibition of the normal ADPG pyrophosphorylase gene in a normal corn line would produce sweetcorn. If normal corn plants are transformed with a partial sense or an antisense ADPG pyrophosphorylase gene construct under the control of a positive or negative gene switch, the crop grower has the ability to switch between production of normal starch or sweetcorn by switching the target gene on or off as desired. With a positive gene switch, sweetcorn is produced in the presence of inducer. With a negative gene switch, sweetcorn is produced in the absence of inducer.

Other sweetcorns existing in nature carry mutations in other starch enzyme genes which disrupt starch synthesis and cause the "sweetcorn" phenotype. For example, the sugary sweetcorn mutant carries a mutation in the soluble starch synthase gene, and the brittle1 sweetcorn mutant (Sullivan et al, 1991, Plant Cell, 3(12):1337-1348; Sullivan et al, 1990, J Cell Biochem Suppl, 0 (14 part E):358) may carry a mutation in the amylogenin gene. Thus switchable or inducible sweetcorn may be produced as hereinbefore described with reference to the ADPG pyrophosphorylase gene using suitable transformation constructs including other target genes.

In a particularly preferred embodiment of the invention there is provided a corn plant comprising a genome lacking an enzyme active in the biosynthesis of starch such that, in its unaltered state, said plant produces seed of the sweetcorn phenotype, said genome further comprising a gene construct, stably incorporated therein by transformation, including a copy of the gene which the unaltered genome lacks, said copy being under the control of a gene switch.

The invention also provides seed corn of the genotype described in the preceding paragraph which, when grown in the presence of the inducer of the gene switch, produces corn plants and seed of normal phenotype and which produces seed of the sweetcorn phenotype in the absence of the inducer.

The method according to the invention may also be used to produce crops with switchable starch yield; that is, with a chemically regulatable capacity to produce an increased/decreased amount of starch and/or to produce starch at a faster/slower rate and/or at a higher/lower temperature optimum. For example, a crop variety may be produced which is adapted to the growth temperature of a particular environment (including particular sites or geographical regions) by having an improved capacity to produce starch at elevated or lowered temperature. If the enzymes genes controlling this capacity may be switched on or off by external control (a chemical inducer), this crop variety may be adapted to more than one temperature regime.

The foregoing discussion is equally applicable to the production of crops with other types of switchable quality (or switchable yield). When the genes responsible for determining quality and/or yield of the crop are identified and isolated, the principles discussed above can be applied to the control of any product's quality or yield. For example, oilseed crops may be transformed with enzyme gene(s) involved in oil production under the control of a gene switch.

The present invention is described, by way of illustration, by the preceding description and following examples with reference to the accompanying drawings of which:

Figure 1 shows the nucleotide sequence for E coli glycogen synthase.

Figure 2 shows the cDNA sequence of wheat leaf ADP-glucose pyrophosphorylase (clone WL.AGA.1).

Figure 3 shows the cDNA sequence of wheat leaf ADP-glucose pyrophosphorylase (clone WE.AGA.3).

Figure 4 shows the cDNA sequence of wheat endosperm ADP-glucose pyrophosphorylase (clone WE.AGA.7).

Figure 5 shows the nucleotide sequence of the GST II-27 gene promoter.

Figure 6 shows the nucleotide sequence of cDNA encoding GST II-27.

Figure 7 shows the construct pZM/RMS-3.

Figure 8 gives an overview of the method of vector construction.

Figure 9 is a graph showing GUS activity in endosperm.

EXAMPLE 1

GST II-27 PROMOTER DRIVES INDUCIBLE EXPRESSION IN ENDOSPERM

The construct pZM/RMS-3 (shown in Figure 7) is a stable transformation vector for Zea mays made using standard techniques. The abbreviations used in Figure 7 are as follows: GST (GST II-27 promoter); GUS (β-glucuronidase reporter gene); NOS (nopaline synthase terminator); 35S (Cauliflower mosaic virus 35S promoter); I1 (maize alcohol dehydrogenase intron 1); BAR (phosphinothricin acyl transferase); TERM (Cauliflower mosaic virus 35S terminator); AMP (prokaryotic ampicillin resistance).

pZM/RMS-3 was constructed using approximately 3.8 Kb of the GST II-27 promoter isolated from plasmid pGIE7 (NCIMB 40426). Nde I was used to cut the GST II-27 promoter at the ATG and 4 Kb upstream. This fragment was cut with EcoRI, blunted and cloned into the SmaI site of pTAK (a Bin19 based promoterless GUS construct) to give pGSTTAK. The GST-GUS-nos cassette from pGSTTAK was then cloned into a pUC derived vector containing the Bar selectable cassette giving pZM/RMS-3. Figure 8 gives an overview of the method of vector construction.

The construct comprises the β-glucuronidase gene (GUS) under the control of the inducible GST II-27 promoter. (For experimental purposes, the GUS gene was used instead of a starch or glycogen biosynthetic enzyme gene because it is a suitable reporter gene for monitoring gene expression).

The construct pZM/RMS-3 was transformed into maize plants using the particle bombardment procedure (Gordon-Kamm et al, 1990, Plant Cell, 2:603-618).

Self-pollinated transgenic maize plants heterozygous with respect to the ZM/RMS-3 construct were treated with safener at 16 or 22 days after pollination (dap). Figure 9 shows the results for individual kernels which were untreated (U) or treated with 10 ml of 20g/l safener by spraying (S) or root drench (RD). GUS gene expression was determined 48 hours after safener treatment.

No GUS expression was observed in endosperm extracts prepared from untreated kernels. Safener spray treatment at 16 dap caused a significant elevation of GUS activity in endosperm. Similarly, spray application and root application of safener at 22 dap caused elevated levels of GUS expression in endosperm. A proportion of the treated kernels show no GUS expression; these represent the azygous progeny from the heterozygous self pollination.

Northern analysis of RNA transcripts showed that uninduced endosperm (14 dap and 24 dap) and uninduced embryo (24 dap) contained no GST II-27 transcript. However, after safener treatment GST II-27 transcripts were significantly elevated in induced endosperm and induced embryo (at 14 dap and 24 dap).

These results clearly show that a gene switch construct may be used to control gene expression in maize endosperm.

EXAMPLE 2

CONSTRUCTION OF PLANT TRANSFORMATION VECTORS

Plant transformation vectors for use in the method of the invention may be constructed using standard techniques. For example, the GUS reporter gene used in construct pZM/RMS-3 (Example 1) may be replaced with the required starch or glycogen biosynthetic enzyme sequence.

2A USE OF GLYCOGEN SYNTHASE

The use of cDNA clones of animal and bacterial glycogen synthases are described in US patent application number 948280 and International patent application publication number GB92/01881. The nucleotide and amino acid sequences of glycogen synthase are known from the literature. For example, Figure 1 shows the nucleotide sequence for the E coli glgA gene encoding glycogen synthase as retrieved from the GenBank/EMBL (SWISS-PROT) database, accession number J02616 (]Kumar et al, 1986, J Biol Chem, 261:16256-16259). E coli glycogen biosynthetic enzyme structural genes were also cloned by Okita et al (1981, J Biol Chem, 256(13):6944-6952). The glycogen synthase glgA structural gene was cloned from Salmonella typhimurium LT2 by Leung et al (1987, J Bacteriol, 169(9):4349-4354). The sequences of glycogen synthase from rabbit skeletal muscle (Zhang et al, 1989, FASEB J, 3:2532-2536) and human muscle (Browner et al, 1989, Proc Natl Acad Sci, 86:1443-1447) are also known.

The most favoured sources of the glycogen synthase gene for use in this invention are bacterial rather than animal sources because:
(1) the bacterial glycogen synthase and plant soluble starch synthase both use ADPG, whereas the animal GS enzyme uses UDPG;
(2) the bacterial GS and plant SSS enzymes do not have any phosphorylation sites for activation, whereas the animal enzyme does; and,
(3) the animal GS enzyme requires glucose-6-phosphate as a co-factor and is

allosterically activated, whereas the plant SSS and bacterial GS enzymes are not.

For these reasons the bacterial GS gene is preferred. The bacterial and animal GS sequences are not homologous. The structural genes for the bacterial GS are mapped to pOP12 in E coli and glycogen synthase maps to glgA. Nucleotide sequencing further refined the position of glgA. The translation start point of glgA is known to be immediately following glgC and the nucleotide sequence determined. The NH_2 sequence was known so that the actual start of the glgA gene was unambiguously determined as well as confirming the direction of transcription. The deduced amino acid sequence shows complete homology with the known NH_2 sequence and with the known amino acid sequence. Different bacterial enzymes show 90% homology. There is complete agreement between the reported and deduced amino acid sequences for the enzyme. Cells transformed with the gene produce a polypeptide that has sequence homology with the known amino acid sequences.

E coli glycogen synthase (Figure 1) is not a large protein: the structural gene is 1431 base pairs in length, specifying a protein of 477 amino acids with an estimated molecular weight of 49,000. It is known that problems of codon usage can occur with bacterial genes inserted into plant genomes but this is generally not so great with E coli genes as with those from other bacteria such as those from Bacillus. Glycogen synthase from E coli has a codon usage profile much in common with maize

genes but it is preferred to alter, by known procedures, the sequence at the translation start point to be more compatible with a plant consensus sequence:

glgA --- G A T A A T G C A G
cons --- A A C A A T G G C T

The GS gene construct requires the presence of an amyloplast transit peptide to ensure its correct localisation in the amyloplast. It is believed that chloroplast transit peptides have similar sequences (Heijne et al describe a database of chloroplast transit peptides in 1991, Plant Mol Biol Reporter, 9(2):104-126). Other potential transit peptides are those of ADPG pyrophosphorylase (1991, Plant Mol Biol Reporter, 9:104-126), small subunit RUBISCO, acetolactate synthase, glyceraldehyde-3P-dehydrogenase and nitrite reductase. For example, the consensus sequence of the transit peptide of small subunit RUBISCO from many genotypes has the sequence:
MASSMLSSAAVATRTNPAQASMVAPFTGLKSAAFPVSRKQNLDITSIASNGGRVQC
and the corn small subunit RUBISCO has the sequence:
MAPTVMMASSATATRTNPAQASAVAPFQGLKSTASLPVARRSSRSLGNVA
NGGRIRC. The transit peptide of leaf starch synthase from corn has the sequence:
MAALATSQLVATRAGLGVPDASTFRRGAAQGLRGARASAAADTLSMRTASA
RAAPRHQQQARRGGRFPSLVVC.
The transit peptide of leaf glyceraldehyde-3P-dehydrogenase from corn has the sequence:
MAQILAPSTQWQMRITKTSPCATPITSKMWSSLVMKQTKKVAHSAKFRVMA
VNSENGT.

The putative transit peptide from ADPG pyrophosphorylase from wheat has the sequence: RASPPSESRAPLRAPQRSATRQHQARQGPRRMC.

2B USE OF BRANCHING ENZYME

The use of cDNA clones of plant and bacterial and animal branching enzymes are described in US patent application number 948280 and International patent application publication number GB92/01881. The nucleotide and amino acid sequences for bacterial branching enzymes (BE) are known from the literature. For example, Kiel et al cloned the branching enzyme gene glgB from Cyanobacterium synechococcus-sp PCC7942 (1989, Gene (Amst), 78(1):9-18) and from Bacillus stearothermophilus (Kiel et al, 1991, Mol Gen Genet, 230(1-2):136-144). The genes glc3 and gha1 of S cerevisiae are allelic and encode the glycogen branching enzyme (Rowen et al, 1992, Mol Cell Biol, 12(1):22-29). Matsumomoto et al investigated glycogen branching enzyme from Neurospora crassa (1990, J Biochem, 107:118-122). The GenBank/EMBL database also contains sequences for the E coli glgB gene encoding branching enzyme.

Branching enzyme [1,4-α-D-glucan: 1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano) transferase (E.C. 2.4.1.18)] converts amylose to amylopectin, (a segment of a 1,4-α-D-glucan chain is transferred to a primary hydroxyl group in a similar glucan chain) sometimes called Q-enzyme. Like soluble starch synthase, this reaction also has temperature-dependent properties in plants,

presumably because of the same molecular mechanisms of helix-to-chain transitions. It is reasonable to believe that the bacterial BE enzyme will behave similarly.

Bacterial branching enzyme genes may be used in this invention, although plant sequences can also be used (rice endosperm: Nakamura et al, 1992, Physiologia Plantarum, 84:329-335 and Nakamura and Yamanouchi, 1992, Plant Physiol, 99:1265-1266; pea: Smith, 1988, Planta, 175:270-279 and Bhattacharyya et al, 1989, J Cell Biochem, Suppl 13D:331; maize endosperm: Singh and Preiss, 1985, Plant Physiology, 79:34-40; Vos-Scherperkeuter et al, 1989, Plant Physiology, 90:75-84; potato: Kossmann et al, 1991, Mol Gen Genet, 230(1-2):39-44; cassava: Salehuzzaman and Visser, 1992, Plant Mol Biol, 20:809-819).

The sequence of maize branching enzyme-I was investigated by Baba et al, 1991, BBRC, 181:87-94. Starch branching enzyme-II from maize endosperm was investigated by Fisher et al (1993, Plant Physiol, 102:1045-1046). We have determined the N-terminal sequences of an 86kD branching enzyme-II from B73 maize as follows:

Ala-Ala-Ala-Arg-Lys-Ala-Val-Met-Val-Pro-Glu-Gly-Glu-Asn-Arg-Glu-Phe-Val-Lys-Tyr-(Leu)-(Phe).....

Thre fragments from this 86kD BEII protein from B73 maize are shown below:

1. ...Val-(Arg)-Pro-Pro-Pro-Xxx-Asp-Gly-Asp-Gly-Ile-Phe-Ile...
2. ...Gln/(Gly)-His-Leu-Xxx-Gln-Tyr-Tyr...

3. ...Ile-Phe-Gln-Ile-Asp-Pro-Met-Leu-Ser-Thr-Tyr-Lys-Tyr...

The BE gene construct may require the presence of an amyloplast transit peptide to ensure its correct localisation in the amyloplast, as discussed previously for the glycogen synthase gene.

2C USE OF SOLUBLE STARCH SYNTHASE

The use of cDNA clones of plant soluble starch synthases are described in US patent application number 948280 and International patent application publication number GB92/01881. The amino acid sequences of pea soluble starch synthase isoforms I and II were published by Dry et al (1991, Plant Journal, 2:193-202). Dry et al later described the characterization and sequence of cDNAs encoding two isoforms of granule bound starch synthase from pea and potato (1992, The Plant Journal, 2(2)). Visser et al described the molecular cloning and partial characterization of the gene for granule-bound starch synthase from potato (1989, Plant Sci (Shannon), 64(2):185-192). Visser et al have also decribed the inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs (1991, Mol Gen Genetic, 225(2):289-296).

The following segments of plant starch synthase (and E coli glycogen synthase) sequences include the sequence KTGGL which is known to be the ADPG binding site:

Pea	GEKPPPLAGTNVMNIILVSAECAPWSKTGGLGDVAGSLPKAL
Maize	ASAGMNVVFVGAEMAPWSKTGGLGDVLGGLP
Barley	ATGSGMNLVFVGAEMAPWSKTGGLGDVLGGLP
Potato	GKGMNLIFVGTEVGPESKTGGLGDVLGGLP
(E coli)	MQVLHVCSEMFPLLKTGGLADVIGALP

2D USE OF ADP GLUCOSE PYROPHOSPHORYLASE

The sequences of bacterial ADPG pyrophosphorylases are known, for example the nucleotide sequence of the E coli glg-C gene (Baecker et al, 1983, J Biol Chem, 258:5084-5088; Leung et al, 1986, J Bacteriol, 167(1):82-88), the glg-C gene from S typhimurium LT2 (Leung et al, 1987, J Bacteriol, 169(9):4349-4354). The GenBank/EMBL database also contains sequences for the E coli glgC gene encoding ADP-glucose pyrophosphorylase.

A review has been published on plant ADP-glucose pyrophosphorylase (Kleczkowski et al, 1991, Journal of Biosciences, 46(7-8);605-612). The isolation and characterisation of cDNA clones for ADPG pyrophosphorylase and waxy locus in wheat was described by Ainsworth amd London during a Symposium on molecular strategies for crop improvement held at the 19th Annual UCLA (University of California-Los Angeles) Symposia on molecular and cellular biology, Keystone, Colorado,

USA, April 16-22, 1990 (J CELL BIOCHEM SUPPL 0 (14 PART E):274). The isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase polypeptides from wheat leaf and endosperm were described by Olive et al, 1989, Plant Mol Biol, 12(5):525-538). The genomic nucleotide sequence of a wild-type shrunken-2 allele of Zea mays was described by Shaw and Hannah (1992, Plant Physiol, 98:1214-1216).

DNA encoding ADP-glucose pyrophosphorylase is described in US patent application number 930935 and European patent application publication number EPA 368506; E coli harbouring plasmids containing such DNA were deposited at the National Collection of Industrial and Marine Bacteria on 19th October 1988 under the Accession Numbers NCIB 40065, NCIB 40066, and NCIB 40067. Figure 2 shows the cDNA sequence of wheat leaf ADP-glucose pyrophosphorylase (clone WL.AGA.1). Figure 3 shows the cDNA sequence of wheat leaf ADP-glucose pyrophosphorylase (clone WE.AGA.3). Figure 4 shows the cDNA sequence of wheat endosperm ADP-glucose pyrophosphorylase (clone WE.AGA.7).

2E USE OF SELF-GLUCOSYLATING PROTEIN

The formation of a glycoprotein primer may be a universal feature for the synthesis of polysaccharides such as starch, glycogen, cellulose etc. The priming molecules may be self-glucosylating proteins (SGP), for example glycogenin which acts as a primer for glycogen synthesis in animals or amylogenin which acts as a primer for starch synthesis in plants (Lomako et

al, 1988, FASEB J, 2:3097-3103 and 1990, FEBS Lett, 268:8-12 and 1991, FEBS Lett, 279:223-228; Cao et al, 1993, J Biol Chem, 268(20):14687-14693; International patent application number GB93/01821). The gene for any such primer may be used in constructs according to this invention.

The glycogenin protein from rabbit skeletal muscle has been sequenced by Campbell and Cohen (1989, Eur J Biochem, 185:119-125), and a glycogenin cDNA has also been identified (Viskupic et al, 1991, FASEB J, 5(6):A1547 and 1992, J Biol Chem, 267(36):25759-25763). The partial amino acid sequencing of peptide fragments of amylogenin from B73 maize was carried out by Gieowar-Singh, Lamoko and Whelan (1992, FASEB J, 6(4):A1520 and A3382); the amino acid sequences of nine purified tryptic peptides (labelled T1-T9) are shown below.

T 1 (P 1) - Y V N A V M T I P K
T 2 (P 3) - E G A N F V X G Y P F S L R *
T 3 (P 4) - Y X X M W A G W T V K
T 4 (P 4) - E G A H T A V S H G L W L N I P D Y D A
P T Q L V K P K
T 5 (P 5) - L G D A M V T W I E A W D E L N P S T P
A A A D G K
T 6 (P 6) - L G D A M V T D I E A A D E L N P A G P
X X X X K
T 7 (P 6) - N L L S P S T P F F F N T L Y D P Y R E
G A N F V X G Y P F S L R *
T 8 (P 7) - G I F W Q E D I I P F F Q N V T I P K
T 9 (P 9) - N L D F L E M W R P F F Q P Y H L I I V
Q D G D P T K

* radioglucosylated tryptic peptides

The use of glycogenin and amylogenin DNA sequences is described in International patent application number GB93/01821. A cDNA clone encoding amylogenin from B73 maize was deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA under the terms of the Budapest Treaty on 19 August 1993 under the accession number ATCC 69389. Partial cDNA sequences corresponding to amylogenin are given below in Tables 1 and 2.

TABLE 1

SEQUENCE (I)

TGAACTTGGCCTTTGACCGTGAGCTCATTGGTCCGGCTATGTACTTCGGTC
TCCTGGGTGATGGTCAGCCTATTGGTCGCTACGACGATATGTGGGCTGGGT
GGTGTGTCAAGGTGATCTGTGATCATTTGGGATTGGGAGTGAAGACGGGTC
TTCCCTACATCTACCACAGCAAGGCGAGCAACCCATTTGTGAACCTGAAGA
AGGAGTACAAGGGAATTTTCTGGCAGGAGGACATCATGCCTTTCTTCCAGA
GTGCAAAGCTCTCGAAAGAAGCTGTGACGGTTCAACAATGCTACATTGAGC
TGTCCAAGATGGTGAAGGAGAAGCTTAGCGCCATTGATCCTTACTTTGACA
AGCTTGCTGATGCTATGGTGACTTGGATTGACGCTTGGGATGTGCTTAACC
CGGCCACATAAG

TABLE 2

SEQUENCE (II)

CTTCCGTTCTTCTTTAACACCTTGTACGATCCCTACCGTGAAGGTGCTGAC
TTCGTCCGTGGATACCCTTTCAGTCTCCGTGAGGGTGTTTCCACTGCTGTT
TCTCACGGTCTCGGGCTCAACATCCCTGATTACGACGCCCCAACTCAACTC
GTCAAGCCTAAGGAAAGAAACACAAGGTATGTGGATGCTGTCATGACCATC
CCAAAGGAACACCTTTGGCCAATTGTGTGGCATGAACTGCC

CLAIMS

1. A method of producing a plant with switchable starch-synthesising ability comprising stably incorporating into the genome of a recipient plant at least one target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and under the control of a gene switch.

2. A method as claimed in claim 1 which produces a plant with switchable starch quality.

3. A method as claimed in either claim 1 or claim 2 which produces a plant with switchable starch yield.

4. A DNA construct which comprises at least one target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and under the control of a gene switch.

5. A DNA construct as claimed in claim 4 in which at least one target gene encodes soluble starch synthase.

6. A DNA construct as claimed in claim 4 in which at least one target gene encodes branching enzyme.

7. A DNA construct as claimed in claim 4 in which at least one target gene encodes glycogen synthase.

8. A DNA construct as claimed in claim 4 in which at least one target gene encodes ADP-glucose pyrophosphorylase.

9. A DNA construct as claimed in claim 4 in which at least one target gene encodes glycogenin.

10. A DNA construct as claimed in claim 4 in which at least one target gene encodes amylogenin.

11. A DNA construct as claimed in claim 4 in which the target gene is derived from cDNA.

12. A DNA construct as claimed in claim 4 in which the target gene is derived from gDNA.

13. A DNA construct as claimed in claim 4 in which at least one target gene is in the sense orientation and encodes all or part of the enzyme.

14. A DNA construct as claimed in claim 4 in which at least one target gene is in the antisense orientation and encodes all or part of the enzyme.

15. A DNA construct as claimed in claim 4 in which at least one target gene encodes a modified allelic form of the enzyme.

16. A DNA construct as claimed in claim 4 in which at least one target gene is preceded by a transit-peptide encoding sequence.

17. A DNA construct as claimed in claim 4 in which the gene switch is a positive gene switch comprising an inducible promoter.

18. A DNA construct as claimed in claim 4 in which the gene switch is a negative gene switch comprising an inducible promoter and a repressor-operator system.

19. A DNA construct as claimed in either claim 17 or claim 18 in which the inducible promoter is the promoter for the 27 kD subunit of glutathione-S-transferase, isoform II.

20. A plant and progeny thereof having at least one target gene stably incorporated into the plant's genome, the or each target gene encoding an enzyme involved in a starch or glycogen biosynthetic pathway and being under the control of a gene switch, such that the plant has switchable starch-synthesising ability.

21. A plant as claimed in claim 20 which is dicotyledonous.

22. A plant as claimed in claim 20 which is monocotyledonous.

23. A plant as claimed in claim 20 which is of the family Gramineae.

24. A plant as claimed in claim 23 which is a maize plant.

25. A maize plant as claimed in claim 24 which is a switchable sweetcorn.

26. A plant as claimed in claim 20 which is a fruit-bearing plant.

27. A plant as claimed in claim 26 which is a tomato plant.

28. Seed of a plant as claimed in claim 20.

29. A plant as claimed in claim 20 with a genome which is homozygous for the target gene or genes encoding the said enzyme.

30. A hybrid plant of which at least one parent is a plant as claimed in claim 29.

31. A method of producing a plant with switchable product-synthesising ability comprising stably incorporating into the genome of a recipient plant at least one target gene encoding an enzyme involved in the product's biosynthetic pathway and under the control of a gene switch.

32. A method as claimed in claim 31 which produces a plant with switchable product quality.

33. A method as claimed in either claim 31 or claim 32 which produces a plant with switchable product yield.

34. A method as claimed in claim 31 in which the product is an oil.

35. A method as claimed in claim 31 in which the product is starch.

36. A corn plant comprising a genome lacking an enzyme active in the biosynthesis of starch such that, in its unaltered state, said plant produces seed of the sweetcorn phenotype, said genome further comprising a gene construct, stably incorporated therein by transformation, including a copy of the gene which the unaltered genome lacks, said copy being under the control of a gene switch.

37. Seed corn produced by a corn plant as claimed in claim 36 which, when grown in the presence of the inducer of the gene switch, produces corn plants and seed of normal phenotype and which produces seed of the sweetcorn phenotype in the absence of the inducer.

FIG.1

gtgattggtgaaaacgcagaggaagatgcacgtcgtttctatcgttcagaagaaggcatcgtgctggtaa
cgcgcgaaatgctacggaagttagggcataaacaggagcgataatgcaggttttacatgtatgttcagag
atgttcccgctgcttaaaccggcggtctggctgatgttattggggcattacccgcagcacaaatcgcag
acggcgttgacgctcgcgtactgttgcctgcatttcccgacattcgccgtggcgtgaccgatgcgcaggt
agtatcccgtcgtgatacctccgccggacatatcacgctgttgttcggtcattacaacggggttggcatt
tacctgattgacgcgccgcatctctatgatcgtccgggaagtccgtatcacgataccaacttatttgtcc
ataccgacaacgtattgcgttttgcgctgctggggtgggttggggcagaaatggccagcgggcttgaccc
attctggcgtcctgatgtggtgcatgcgcacgactggcatgcaggccttgcgcctgcgtatctggcggcg
cgcgggcgtccggcgaagtcggtgtttactgtgcacaacctagcctatcaaggcatgttttatgcacatc
acatgaatgacatccaattgccatggtcattctttaatattcatggctggaattcaacggacaaatctc
tttcctgaaggccggtctgtactatgccgatcacattacggcggtcagtccaacctacgctcgcgagatc
accgaaccgcagtttgcctacggtatggaaggtctgttgcaacagcgtcaccgcgaagggcgtctttccg
gcgtaccgaacggcgtggacgagaaaatctggagtccagagacggacttactgttggcctcgcgttacac
ccgcgatacgttggaagataaagcggaaaataagcgccagtcacaaatcgcaatgggatccaaggttgac
gataaagtgccgcttttgcagtggtgagccgtctgaccagccagaaaggtctcgattcggtgctggaag

cctcaccgggttcttcggagcagggcgggcagctggcgctactcggcgcgggcgatccggtgctgcagga
aggtttccttgcggcggcagcggaataccccggtcaggtgggcgttcagattggctatcacgaagcattt
tcgcatcgcattatgggcggcgcggacgtcattctggtgcccagccgtttcgaaccgtgcggcttaacgc
aactttatggatcgaagtacggtacgctgccgttagtgcgacgcaccggtgggcttgctgatacggtttc
tgactgttctctcgagaaccttgcagatggcgtcgccaatgggtttatcttcgaagatagtaatgcctgg
tcgctgttacggactattcgacgtgcttttgtactgtggtcctgtcctccactgtggcggtttgtgcaac
gtcaggctatggcaatggatttggctggcaggtcgcggcgaagtcgtaccgtgagctttactatcgctc
gaaatagttttcaggaaacgcctacatgaatgctccgtttacatattcatcgcccacgctt1

A) WL : AGA.1

FIG. 2

```
G V L I L S G D H L Y R M D Y M D F V Q S H R Q R D A G I S I C C L P I D G S R
GGGGTGCTGATTCTTTCTGGCGATCACCTCTACCGTATGGACTACATGGATTTTGTTCAGAGTCATCGGCAGAGAGACGCGGGGATCAGCATCTGTTGCTTGCCTATTGATGGCAGCCGG
      10        20        30        40        50        60        70        80        90        100       110       120

A S D F G L M K I D D T G R V I S F S E K P R G A D L K E M E E A E K K P Y I A
GCGTCTGATTTTGGTCTCATGAAGATAGACGACACAGGAAGAGTTATTTCATTTAGTGAAAAACCGAGAGGAGCTGATTTAAAGGAAATGGAGGAAGCAGAAAAGAAACCATACATAGCT
      130       140       150       160       170       180       190       200       210       220       230       240

S M G V Y I F K K E I L L N L L R H R F P T A N D F G S E I I P A A A R E I N V
TCAATGGGAGTATACATATTCAAGAAAGAGATACTTCTAAATCTTTTGAGATGGCGTTTTCCCACTGCAAATGATTTTGGATCTGAAATAATTCCAGCTGCAGCAAGAGAGATTAATGTA
      250       260       270       280       290       300       310       320       330       340       350       360

K A Y L F N D Y H E D I G T I K S F F E A N L A L A E Q P S K F S F Y D A S K P
AAGGCATATCTTTTCAATGATTACTGGGAAGATATTGGAACTATCAAATCCTTCTTCGAAGCAAATCTTGCCCTTGCTGAACAGCCTTCAAAGTTCAGCTTCTATGATGCTAGCAAACCG
      370       380       390       400       410       420       430       440       450       460       470       480

M Y T S R R N L P P S M I S G S K I T D S I I S H G C F L D K C R V E H S V V G
ATGTACACATCGCGAAGAAACCTACCACCATCTATGATCAGCGGTAGTAAGATCACTGATTCGATCATTTCCCATGGATGTTTCTTGGATAAATGCAGGGTAGAGCACAGTGTCGTTGGA
      490       500       510       520       530       540       550       560       570       580       590       600

I R S R I G S N V H L K D T V M L G A D F Y E T D M E R G D Q L A E G K V P I G
ATCCGTTCTCGAATAGGCTCCAACGTACACCTCAAGGATACGGTAATGCTCGGTGCTGATTTCTATGAAACTGACATGGAAAGAGGCGACCAGCTGGCCGAAGGAAAGGTTCCGATTGGG
      610       620       630       640       650       660       670       680       690       700       710       720

I G E N T S I Q N C I I D K N A R I G K N V T I A N A E G V Q E A D R A S E G F
ATCGGGGAGAACACTTCGATTCAAAACTGCATCATTGACAAGAATGCGAGGATAGGGAAGAATGTGACCATTGCTAACGCCGAGGGTGTACAGGAAGCGGACAGGGCGTCAGAAGGCTTC
      730       740       750       760       770       780       790       800       810       820       830       840

H I R S G I T V V L K N S V I A D G L V I
CACATCCGGTCCGGTATCACGGTTGTGCTGAAGAACTCGGTGATTGCGGATGGATTAGTCATATGAGCTGAAAAAAGGCGGTTCTCCAGTCCAGCAAGAGAAATAAA
      850       860       870       880       890       900       910       920       930       940
```

FIG. 3 (1/2)

B) WE : AGA.3

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                E  L  V  Q  K  H  V  D  D  N  A  D  I  T  L  S  C  A  P  V  G  E  S  R  A  S  E  Y  G  L  V  K  F  D  S  S  G  R  V  V
CGGAGCTTGTGCAGAAACATGTGGATGACAATTACTTTATCATGTGCCCTGTTGGAGAGAGCCGGGCATCTGAGTACGGGCTAGTGAAGTTCGACAGTTCAGGCCGTGTGG
       10        20        30        40        50        60        70        80        90       100       110       120

                Q  F  S  E  K  P  K  G  D  D  L  E  A  M  K  V  D  T  S  F  L  N  F  A  I  D  D  P  A  K  Y  P  Y  I  A  S  H  G  V  Y
TCCAATTTTCTGAGAAGCCAAAGGGTGACGATCTGGAAGCGATGAAAGTGGACACCAGTTTTCTCAATTTCGCCATCGACGACCCTGCTAAATATCCATACATTGCTTCTATGGGAGTCT
      130       140       150       160       170       180       190       200       210       220       230       240

                V  F  K  R  D  V  L  L  L  N  L  L  K  S  R  Y  A  E  L  H  D  F  G  S  E  I  L  P  R  A  L  H  D  H  N  V  Q  A  Y  V  F
ATGTCTTCAAAAGAGATGTTCTGCTCAACCTTCTAAAGTCAAGATACGCAGAACTACATGACTTTGGTCTGAAATCCTCCCGAGAGCTCTGCATGACCACACAATGTACAGGCTTATGTCT
      250       260       270       280       290       300       310       320       330       340       350       360

                T  D  Y  H  E  D  I  G  T  I  R  S  F  F  D  A  N  H  S  L  C  E  Q  P  P  K  F  E  F  Y  D  P  K  T  P  F  F  T  S  P
TCACTGACTACTGGGAGGACATTGGAACAATCAGATCCTTCTTCGATGCGAACATGTCCCTCTGCGAGCAGCCCCCAAAGTTCGAGTTTTATGATCCCAAAACTCCCTTCTTCACCTCGC
      370       380       390       400       410       420       430       440       450       460       470       480
```

```
                R  Y  L  P  P  T  K  S  D  K  C  R  I  K  E  A  I  I  L  H  G  C  F  L  R  E  C  K  I  E  H  S  I  I  G  V  P  S  R  L
CTCGATACTTGCCACCAACAAACAAAGTCAGACAAGTGCAGGATCAAAGAAGCGATCATTCTGCACGGCTGCTTCTTGCGTGAATGCAAAATCGAGCACTCCATCATCGGCGTTCCTTCACGCC
         490        500        510        520        530        540        550        560        570        580        590        600

   N  S  G  S  E  L  K  N  A  H  M  H  G  A  D  S  Y  E  T  E  D  E  I  S  R  L  H  S  E  G  K  V  P  I  G  V  G  E  N  T
TAAACTCCGGAAGCGAACTCAAGAACGCGATGATGGTGCGGATTCGTACGAGACCGAGCATGAGATCTCGAGGCTGATGTCCGAGGGCAAGGTCCCCATCGGCGTCGGGGAGAACA
         610        620        630        640        650        660        670        680        690        700        710        720

   K  I  S  N  C  I  I  D  M  N  A  R  I  G  R  D  V  V  I  S  N  K  E  G  V  Q  E  A  D  R  P  E  E  G  Y  Y  I  R  S  G
CAAAGATCAGCAACTGCATCATCGACATGAACGCGAGGATAGGAAGGGACGTGGTCATCTCAAACAAGGAGGGAGTGCAAGAAGCCGACAGGCCGGAGGAGGGGTACTACATCAGGTCCG
         730        740        750        760        770        780        790        800        810        820        830        840

      I  V  V  I  Q  K  N  A  T  I  K  D  G  T  V  V
GGATCGTGGTGATCCAGAAGAACGCGACCATCAAGGACGGCACCGTCGTGTAGTACCCGGGTCGGCGTGACGGGTTCTGCGACAACCTCTCGCTGCGTTGATCGTCGTCGTCTCGAG
         850        860        870        880        890        900        910        920        930        940        950        960

GCCCGGGAGGGACTGAAGAAGTGACCGGGGACGGGAGGCGTTTGAAGCTTGAATGACTGAGAAGGCGCGCGCGGGCAGCATTAGTAGTAAGTAGTAAGTAAGGAGCAGTGGAACAAAGTAA
         970        980        990       1000       1010       1020       1030       1040       1050       1060       1070       1080

TAGTCGTTCGTTTTTCCCCTGTAATAAATAAGAGGCTGTGTTGAGGTAAAGGAAGTGGCAGCGAGCGAACAAACAAACTCCCGGGGGATGTTCGTGTAAATAAAACTCTATCTAGACCTGTGA
        1090       1100       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200

AATTTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
        1210       1220       1230       1240       1250       1260       1270
```

FIG. 3(2/2)

FIG. 4 (1 / 3)

C) WE : AGA.7

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                                                                  ↓
R  A  S  P  P  S  E  S  R  A  P  L  R  A  P  Q  R  S  A  T  R  Q  H  Q  A  R  Q  G  P  R  R  H  C  N  G  G  R  G  P  P
CGTGCGTCTCCCCCGTCAGAGTCGAGGCTCCGCTGCGAGCGCCTCAAAGGTCGGCGACACGGCAGCATCAGGCACGACAGGTCCCAGGAGGATGTGCAACGGCGGCAGGGCCCGCCA
      10        20        30        40        50        60        70        80        90        100       110       120

Y  H  T  A  G  V  T  S  A  P  A  R  Q  T  P  L  F  S  G  R  P  S  G  G  L  S  D  P  N  E  V  A  A  V  I  L  G  G  G  T
TACTGGACAGCTGGTGTCACCTCCGGCCCAGCCCGGCAGACACCCTTGTTCTCCGGACGTCCTTCAGGAGGATTAAGCGATCCGAACGAGGTTGCGGCCGTCATACTCGGCGGCGGCACC
      130       140       150       160       170       180       190       200       210       220       230       240

G  T  Q  L  F  P  L  T  S  T  R  A  T  P  A  V  P  I  G  G  C  Y  R  L  I  D  I  P  M  S  N  C  F  N  S  G  I  N  K  I
GGGACTCAGCTCTTCCCACTCACGAGCACAAGGCCACACCTGCTGTTCCTATTGGAGGATGTTACAGGCTCATCGACATTCCCATGAGCAACTGCTTCAACAGTGGCATCAACAAGATA
      250       260       270       280       290       300       310       320       330       340       350       360

F  V  M  T  Q  F  N  S  A  S  L  N  R  H  I  H  R  T  Y  L  G  G  G  I  N  F  T  D  G  S  V  E  V  L  A  A  T  Q  M  P
TTCGTCATGACCCAGTTCAACTCGGCCTCCCTTAATCGTCACATTCACCGCACCTACCTCGGCGGGGGAATCAATTTCACTGATGGATCCGTTGAGGTATTGGCCGCGACGCAAATGCCC
      370       380       390       400       410       420       430       440       450       460       470       480

G  E  A  A  G  W  F  R  G  T  A  D  A  W  R  K  I  I  W  V  L  E  D  Y  Y  K  N  K  S  I  E  H  I  L  L  S  G  D  Q
GGGGAGGCTGCTGCATGGTTCCGCGGAACAGCGGACGCGTGGAGAAAAATTATCTGGGTGCTTGAGGACTATTATAAGAATAAAATCCATAGAGCACATTTTGATCTTGTCGGCGATCAG
      490       500       510       520       530       540       550       560       570       580       590       600
```

FIG. 4 (2/3)

```
L Y R M D Y M E L V Q K H V D D N A D I T L L S C A P V G E S R A S E Y G L V K F
CTTTATCGCATGGATTACATGGAGCTTGTGCAGAAACATGTGGATGACAATGCTGACATTACTTTATCATGTGCCCTTGTTGGAGAGAGCCGGGCATCTGAGTACGGGCTAGTGAAGTTC
      610       620       630       640       650       660       670       680       690       700       710       720

D S S G R V V Q F S E Q P K G D D L E A M K V D T S F L N F A I D D P A K Y P Y
GACAGTTCAGGCCGTGTGGTCCAGTTTTCTGAGCAGCCAAAGGGTGACGATCTGGAAAGTGGACACCAGTTTTCTCAATTTCGCCATCGACGATCCTGCTAAATATCCATAC
      730       740       750       760       770       780       790       800       810       820       830       840

I A S M G V Y V F K R D V L L N L L K S R Y A E L H D F G S E I L P R A L H D H
ATTGCTTCTATGGGAGTCTATGTCTTCAAAAGAGATGTTCTGCTCAACCTTCTAAAGTCAAGATATGCAGAACTACATGACTTTGGGTCTGAGATCCTCCCGAGAGCTCTGCATGACCAC
      850       860       870       880       890       900       910       920       930       940       950       960

N V Q A Y V F T D Y M E D I G T I R S F F D A N R A L C E Q P P K F E F Y D P K
AATGTACAGGCTTATGTCTTCACTGACTACATGGAGGACATTGGAACAATCAGATCCTTCTTCGATGCAAACAGGGCCCTCTGCGAGCAGCCCCCAAAGTTCGAGTTTTATGATCCCAAA
      970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080

T P F F T S P R Y L P P T K S D K C R I K E A I I L H G C F L R E C K I E H T A
ACTCCCTTCTTCACCTCGCCTCGATACTTGCCACCAACAAAGTCAGGACAAGTGCAGGATCAAAGGAAGCGATCATTCTGCACGGCTGCTTCTTGCGTGAATGCAAAATCGAGCACACTGCG
     1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
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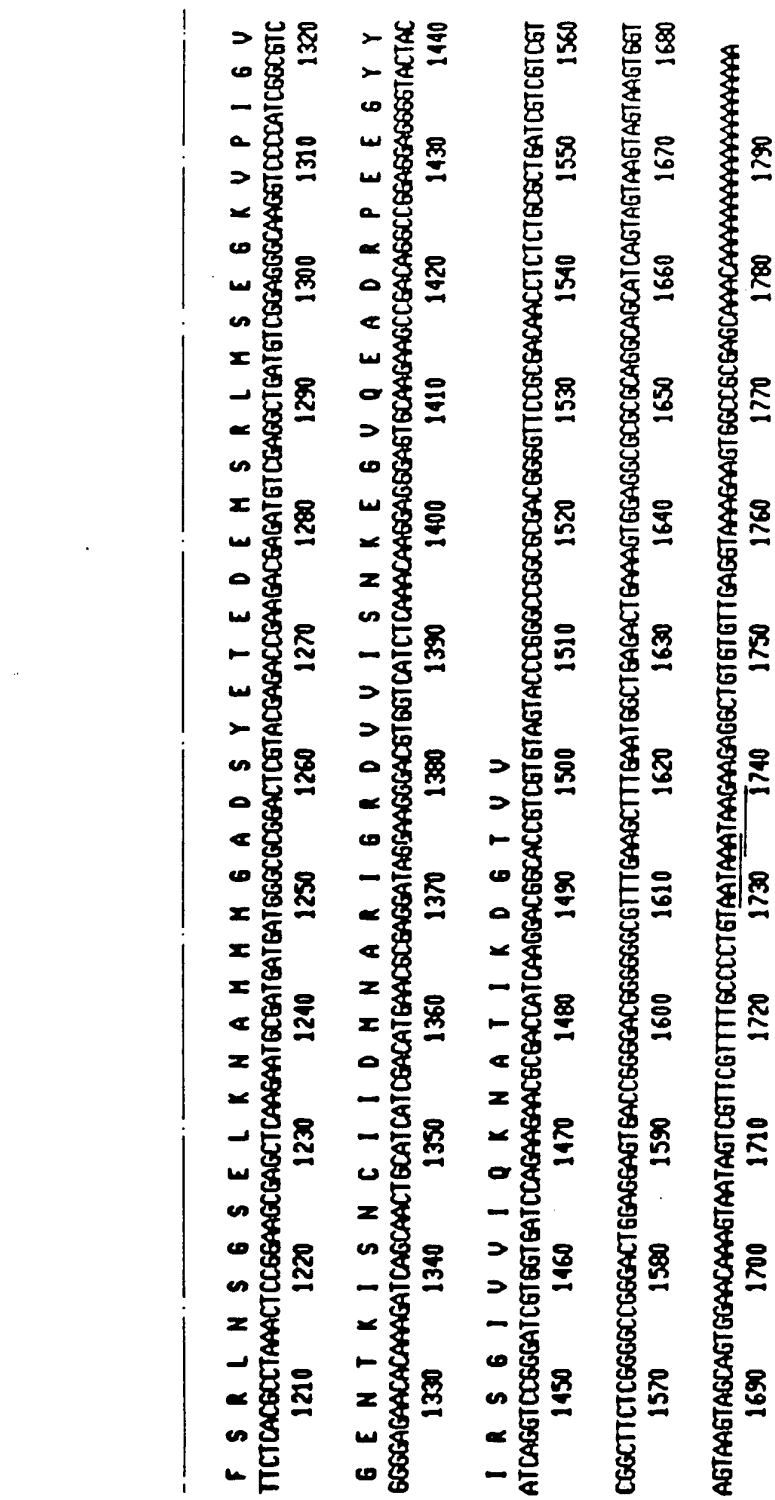
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1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
G E N T K I S N C I I D M N A R I G R D V V I S N K E G V Q E A D R P E E G Y Y
GGGGAGAACACAAAGATCAGCAACTGCATCATCGACATGAACGCGAGGATAGGAAGGGACGTGGTCATCTCAAACAAGGAGGGAGTGCAAGAAGCCGACAGGCCGGAGGAGGGGTACTAC
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
I R S G I V V I Q K N A T I K D G T V V
ATCAGGTCCGGGATCGTGGTGATCCAGAAGAACGCGACCATCAAGGACGGCACCGTCGTGTAGTACCCGGGCCGGCGCGACGAGGGTTCCGCGACAACCTCTCTGCGCTGATCGTCGTCGT
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
CGGCTTCTCGGGGCCGGGACTGGAGGAGTGACCGGGGACGGGGGCGTTTGAAGCTTTGAATGGCTGAGACTGAAAGTGGAGGCGCGCGACGGCAGCATCAGTAGTAAGTAAGTAAGTGGT
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
AGTAAGTAGCAGTGGAACAAAAGTAAATAGTCGTTCGTTTTGCCCCTGTAATAAATAAGAAGAGGCTGTGTGTTGAGGTAAAGAAGTGGCCGCGAGCAAACAAAAAAAAAAAAAAAAAAAAA
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790

FIG. 4 (3/3)

FIG. 5 (1/3)

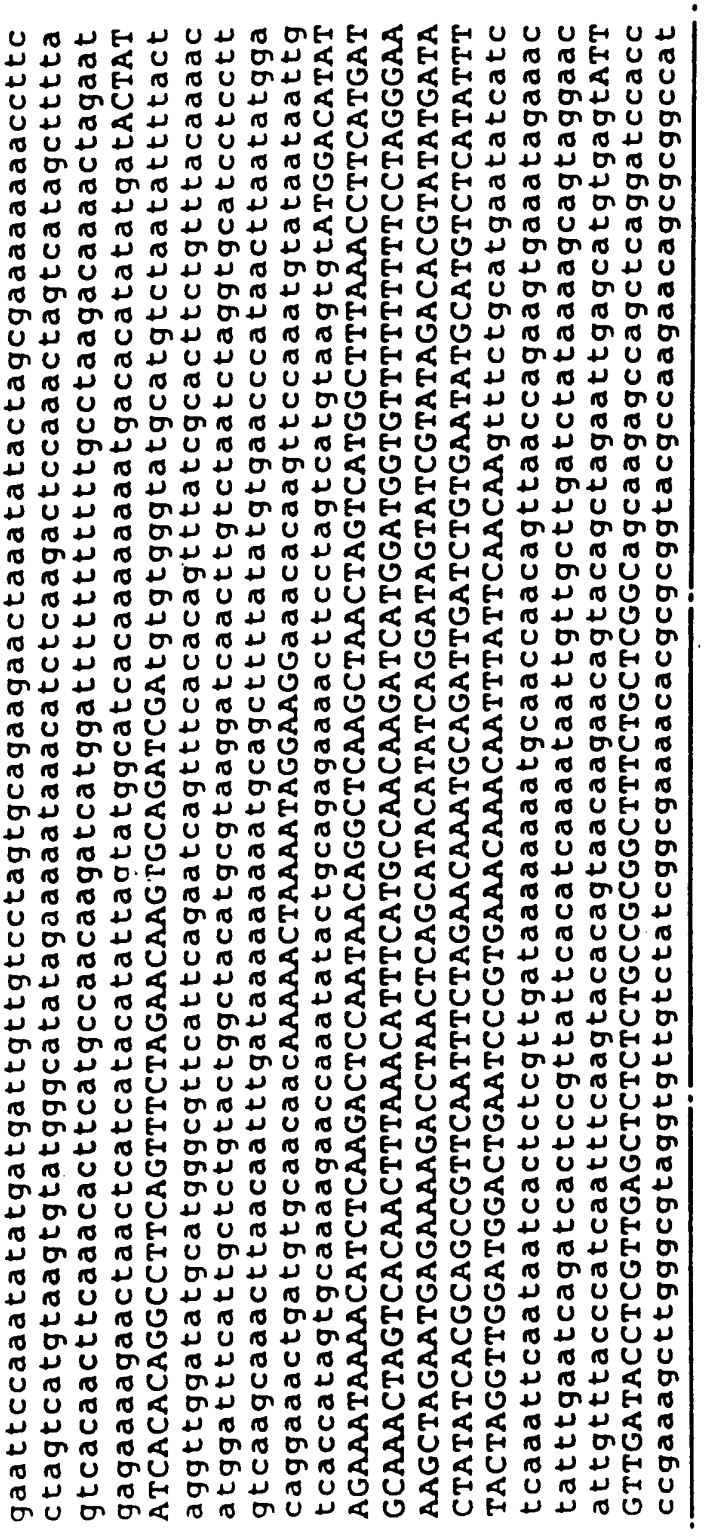
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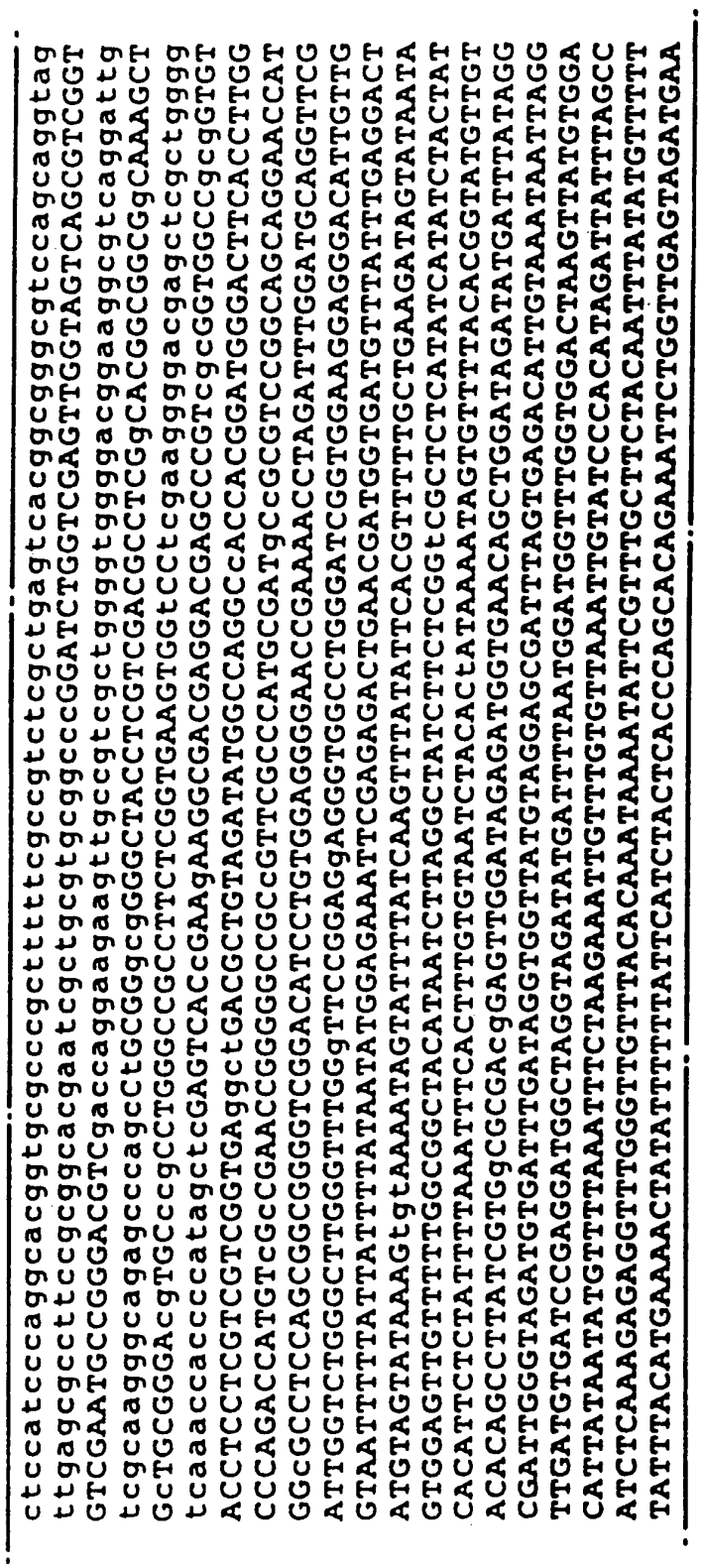
FIG. 5 (2/3)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tcgcaagggcagagcccagcctGCGGgcgGGGCTACCTCGTCGACGCCTCGgCACGGCGGCGGCAAAGCT
GcTGCGGGACgTGCccgCCTGGGCCGCCTTCTCGGTGAAGTGGTcCCtcgaagggggacgagctcgctgggg
tcaaaccaccccatagctcGAGTCACCgGAAgAAGGCGACGAGGACGAGCCCGTcgcGGTGGCCCgcgGTGT
ACCTCCTCGTCGTCGGTGAggctGACGCTGTAGATATGGCCAGGCcACCACGGATGGGACTTCACCTTGG
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GGcGCCTCCAGCGGCGGGGTCGGACATCCTGTGGAGGGGAACCGAAAACCCTAGATTTGGATGCAGGTTCG
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TTGATGTGATCCGAGGATGGCTAGGTAGATATGATTTTAATGGATGGTTTGGTGGACTAAGTTATGTGGA
CATTATAATATGTTTTTAAATTTCTAAGAAATTGTTTGTGTTAAATTGTATCCCACATAGATTATTTAGCC
ATCTCAAAGAGAGGTTTGGTTGTTTACACAAATAAAATATTCGTTTGCTTCTACAATTTATATGTTTTT
TATTTACATGAAAACTATATTTTTTATTCATCTACTCACCCAGCACAGAAATTCTGGTTGAGTAGATGAA

FIG. 5 (3/3)

```
AAAAAACTACAACAAACTCTTCCTGAAAGTGTCGGTGTGAAGCCGAGAAATCCTTTTCATTTCGGTGACG
GAGCCCCCTTGCTGGCTGCTCAGTGCACTCCCGTTCGCCTGCCTGCCACTACAAGCGACGGCCGACGAC
TCGCAAGTATCGGTAGGCATTTTAAAACTGAAAACCAAATCTAAACCCGAATAGACCAAATTGTTGGTTT
ATTCGGGTTTTTGGGTTCGGATTCGGTTTCTAAATATGCTATATTTTTAGGGTATAGGTTCGGGTTCAGTT
TCTAACCTTTAAAACCTGAATAGACGAATAACCCGAAATATAAAAAATCTCTTAATATGTGATGATATTA
TTATATGATTTATTATGAACTTATTAACCGAAAATAATGATACCATCCTAACGATAGTATATATATCTATGTA
TGCTATTTTTTATAGTCACTTGTTGTAATAATAGTACTTCCAATTAATTAATCAGTGTATATATTTTTAACA
AAAGATACTAGCCTCTCTACTATTTGAGTATATTCGGTGCACCGAATAGACCGAACCGAAATTGTAAGTC
TATTCAGGTTCGGTTCCTAAAATTATTTTTAAAAATTTTGGTTCTCATATTTCAGAATCCGAAATTTCATA
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TAGCCTGCTGCGTGCGTAAGCGAGGACGTCACGCGTTCTCCCTCCCGTCGACCAAATACACTTGGTCTTC
TAGCACCTTCTTCCTCTCCAAGaCTCCAATCCCCCAACCACCAGAACCAGCGCCCagCTctAACGTCACCT
CTGATTTCTCTCTCCTCTCTATTGCTAGCTGCTTTATTATAAGTAGCAGCTGCAGGCAGGAGCTGCA
CACACCCATCCAATTCCAGCTGCTGATCTTGATCCTGCACCCCCGAGCCGTACACACAAGAGCTAGTCGGTAG
AAcTTGCAGGAGCGGAgcAGAACTAAGTGCAGAGAACAGGACATATG
                                                  |
                                    translation start point
```

FIG. 6 (1 / 3)

```
                10                  20                  30                  40                  50
                 |                   |                   |                   |                   |
   1 CCAGCTGCTG ATCTTGATCC TGCACCCCGA GCCGTACACA AGAGCTAGTC
     GGTCGACGAC TAGAACTAGG ACGTGGGGCT CGGCATGTGT TCTCGATCAG

  51 GGTAGAACTT GCAGGAGCGG AGCAGAACTA AGTGCAGAGA ACAGGACATA
     CCATCTTGAA CGTCCTCGCC TCGTCTTGAT TCACGTCTCT TGTCCTGTAT

 101 TGGCTACGCC GGCGGTGAAG GTTTACGGGT GGGCTATCTC GCCGTTCGTA
     ACCGATGCGG CCGCCACTTC CAAATGCCCA CCCGATAGAG CGGCAAGCAT

 151 TCGCGGGCTC TGCTGGCCCT GGAGGAGGCC GGCGTCGACT ACGAGCTCGT
     AGCGCCCGAG ACGACCGGGA CCTCCTCCGG CCGCAGCTGA TGCTCGAGCA

 201 CCCCATGAGC CGCCAGGACG GCGACCACCG CCGCCCGGAG CACCTCGCCA
     GGGGTACTCG GCGGTCCTGC CGCTGGTGGC GGCGGGCCTC GTGGAGCGGT

 251 GGAACCCTTT CGGGAAGGTG CCGGTGCTCG AGGATGGCGA CCTCACGCTC
     CCTTGGGAAA GCCCTTCCAC GGCCACGAGC TCCTACCGCT GGAGTGCGAG

 301 TTCGAATCAC GTGCGATCGC GAGGCATGTT CTCCGGAAGC ACAAGCCGGA
     AAGCTTAGTG CACGCTAGCG CTCCGTACAA GAGGCCTTCG TGTTCGGCCT

 351 GCTGCTGGGC GGCGGCAGGC TGGAGCAGAC GGCGATGGTG GACGTGTGGC
     CGACGACCCG CCGCCGTCCG ACCTCGTCTG CCGCTACCAC CTGCACACCG

 401 TGGAGGTGGA GGCCCACCAG CTGAGCCCGC CGGCGATCGC CATCGTGGTG
     ACCTCCACCT CCGGGTGGTC GACTCGGGCG GCCGCTAGCG GTAGCACCAC
```

FIG. 6 (2/3)

```
401  TGGAGGTGGA GGCCCACCAG CTGAGCCCGC CGGCGATCGC CATCGTGGTG
     ACCTCCACCT CCGGGTGGTC GACTCGGGCG GCCGCTAGCG GTAGCACCAC

451  GAGTGCGTGT TCGCGCCGTT CCTGGGCCGC GAGCGCAACC AGGCGGTGGT
     CTCACGCACA AGCGCGGCAA GGACCCGGCG CTCGCGTTGG TCCGCCACCA

501  GGACGAGAAC GTGGAGAAGC TCAAGAAGGT GCTGGAGGTG TACGAGGCGC
     CCTGCTCTTG CACCTCTTCG AGTTCTTCCA CGACCTCCAC ATGCTCCGCG

551  GGCTGGCCAC GTGCACGTAC CTCGCCGGCG ACTTCCTCAG CCTCGCCGAC
     CCGACCGGTG CACGTGCATG GAGCGGCCGC TGAAGGAGTC GGAGCGGCTG

601  CTCAGCCCCT TCACCATCAT GCACTGCCTC ATGGCCACCG AGTACGCCGC
     GAGTCGGGGA AGTGGTAGTA CGTGACGGAG TACCGGTGGC TCATGCGGCG

651  TCTCGTCCAT GCGCTCCCGC ACGTCAGCGC CTGGTGGCAG GGCCTCGCCG
     AGAGCAGGTA CGCGAGGGCG TGCAGTCGCG GACCACCGTC CCGGAGCGGC

701  CGCGCCCGGC GGCCAACAAG GTGGCGCAGT TCATGCCGGT CGGCGCCGGA
     GCGCGGGCCG CCGGTTGTTC CACCGCGTCA AGTACGGCCA GCCGCGGCCT

751  GCGCCCAAGG AACAGGAGTG ACGATGAAGC GATCGAAGCG ACTTGTGTTG
     CGCGGGTTCC TTGTCCTCAC TGCTACTTCG CTAGCTTCGC TGAACACAAC
```

```
801 TTGTGCTTGA TTAGTTAATT GGAAACCTTC TCACTCATCT AGTCCATCAT
    AACACGAACT AATCAATTAA CCTTTGGAAG AGTGAGTAGA TCAGGTAGTA

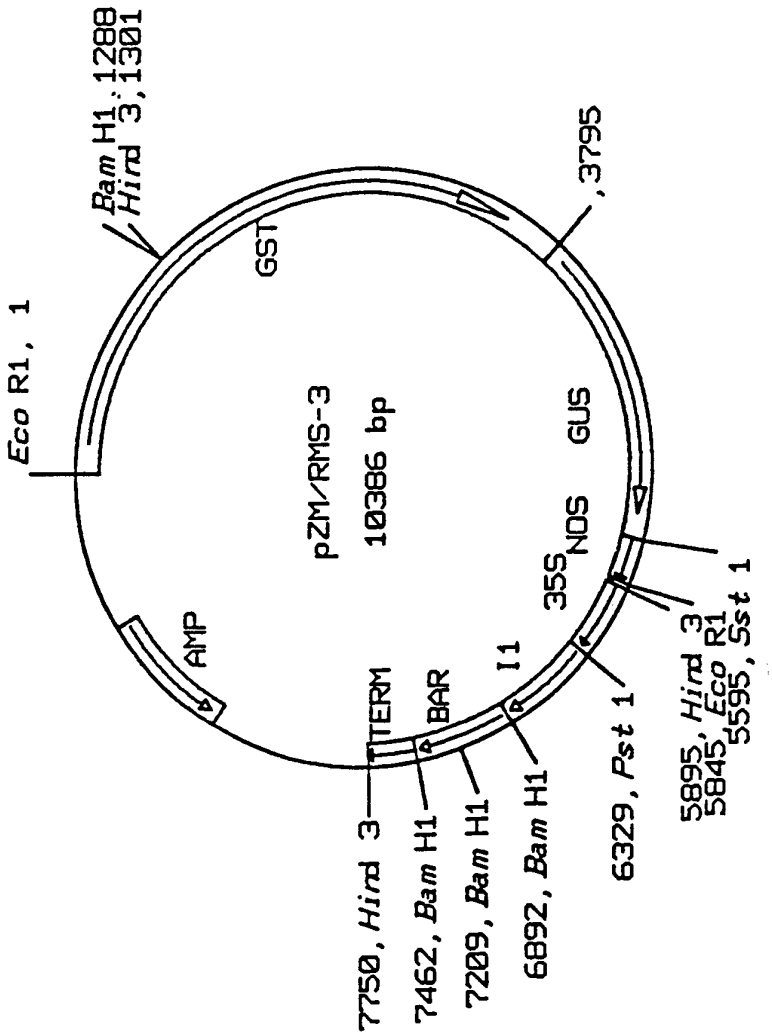
851 GGTGCCTGCT TTTCTTTATA CTATTTGTCT TAATTTTGCT GCTTTCTCCA
    CCACGGACGA AAAGAAATAT GATAAACAGA ATTAAAACGA CGAAAGAGGT

901 CGGAATAATA GTAGAGATTT GGAAATGTAA TGTATTTATC AAAAAAAAAA
    GCCTTATTAT CATCTCTAAA CCTTTACATT ACATAAATAG TTTTTTTTTT

951 AAAA
    TTTT
```

FIG.6 (3/3)

FIG. 7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Bam H1, 1288
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GUS
NOS
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I1
AMP
TERM
BAR
7750, Hind 3
7462, Bam H1
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6892, Bam H1
6329, Pst 1
5895, Hind 3
5845, Eco R1
5595, Sst 1

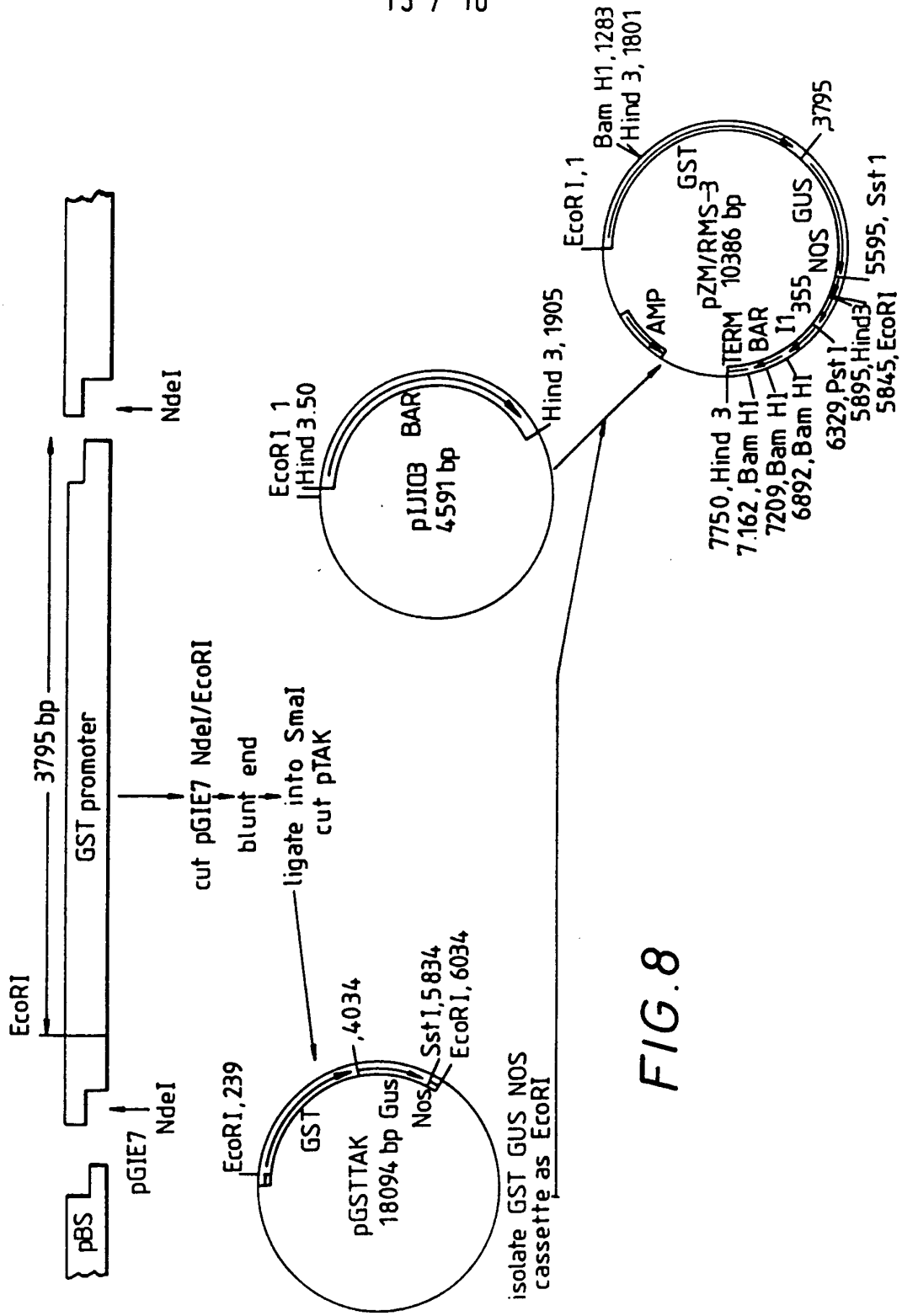
EcoRI
3795 bp
GST promoter
NdeI
pBS
pGIE7
NdeI
cut pGIE7 NdeI/EcoRI
blunt end
ligate into SmaI
cut pTAK
EcoRI, 239
GST
,4034
Sst1,5834
EcoRI, 6034
pGSTTAK
18094 bp Gus
Nos
isolate GST GUS NOS
cassette as EcoRI
EcoR1 1
Hind 3.50
pIJI03 BAR
4591 bp
Hind 3, 1905
EcoR1, 1
Bam H1, 1283
Hind 3, 1801
AMP
pZM/RMS-3
10386 bp
GST
3795
GUS
NOS
355
I1
BAR
TERM
7750, Hind 3
7.162, Bam H1
7209, Bam H1
6892, Bam H1
6329, Pst1
5895, Hind3
5845, EcoRI
5595, Sst1

FIG. 8

FIG. 9 GUS activity in endosprem after safener root drench/spray