MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT

The subject invention pertains to novel variants of the maize gene, Shrunk2(Sh2) and a method of using that gene. The variant gene, Sh2-m1Rev6, encodes a subunit of the ADP-glucose pyrophosphorylase (AGP) enzyme that has additional amino acids inserted in or near the allosteric binding site of the protein. Corn seed expressing the Sh2-m1Rev6 gene has a 15% weight increase over wild type seed. The increase in seed weight is not associated simply with an increase in percentage starch content of the seed.
<table>
<thead>
<tr>
<th>Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
</tr>
<tr>
<td>AM</td>
</tr>
<tr>
<td>AT</td>
</tr>
<tr>
<td>AU</td>
</tr>
<tr>
<td>AZ</td>
</tr>
<tr>
<td>BA</td>
</tr>
<tr>
<td>BB</td>
</tr>
<tr>
<td>BE</td>
</tr>
<tr>
<td>BF</td>
</tr>
<tr>
<td>BG</td>
</tr>
<tr>
<td>BI</td>
</tr>
<tr>
<td>BR</td>
</tr>
<tr>
<td>BY</td>
</tr>
<tr>
<td>CA</td>
</tr>
<tr>
<td>CF</td>
</tr>
<tr>
<td>CG</td>
</tr>
<tr>
<td>CH</td>
</tr>
<tr>
<td>CI</td>
</tr>
<tr>
<td>CM</td>
</tr>
<tr>
<td>CN</td>
</tr>
<tr>
<td>CU</td>
</tr>
<tr>
<td>CZ</td>
</tr>
<tr>
<td>DE</td>
</tr>
<tr>
<td>DK</td>
</tr>
<tr>
<td>EE</td>
</tr>
<tr>
<td>SI</td>
</tr>
<tr>
<td>SK</td>
</tr>
<tr>
<td>SN</td>
</tr>
<tr>
<td>SZ</td>
</tr>
<tr>
<td>TD</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>TJ</td>
</tr>
<tr>
<td>TM</td>
</tr>
<tr>
<td>TR</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>UA</td>
</tr>
<tr>
<td>UG</td>
</tr>
<tr>
<td>US</td>
</tr>
<tr>
<td>UZ</td>
</tr>
<tr>
<td>VN</td>
</tr>
<tr>
<td>YU</td>
</tr>
<tr>
<td>ZW</td>
</tr>
</tbody>
</table>
DESCRIPTION

MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT

This invention was made with government support under National Science Foundation grant number 93052818. The government has certain rights in this invention.

Cross-Reference to a Related Application

This application is a continuation-in-part of co-pending application Serial No. 08/299,675, filed September 1, 1994.

Background of the Invention

ADP-glucose pyrophosphorylase (AGP) catalyzes the conversion of ATP and \( \alpha \)-glucose-1-phosphate to ADP-glucose and pyrophosphate. ADP-glucose is used as a glycosyl donor in starch biosynthesis by plants and in glycogen biosynthesis by bacteria. The importance of ADP-glucose pyrophosphorylase as a key enzyme in the regulation of starch biosynthesis was noted in the study of starch deficient mutants of maize (Zea mays) endosperm (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). AGP enzymes have been isolated from both bacteria and plants. Bacterial AGP consists of a homotetramer, while plant AGP from photosynthetic and non-photosynthetic tissues is a heterotetramer composed of two different subunits. The plant enzyme is encoded by two different genes, with one subunit being larger than the other. This feature has been noted in a number of plants. The AGP subunits in spinach leaf have molecular weights of 54 kDa and 51 kDa, as estimated by SDS-PAGE. Both subunits are immunoreactive with antibody raised against purified AGP from spinach leaves (Copeland and Preiss, 1981; Morell et al., 1987). Immunological analysis using antiserum prepared against the small and large subunits of spinach leaf showed that potato tuber AGP is also encoded by two genes (Okita et al., 1990). The cDNA clones of the two subunits of potato tuber (50 and 51 kDa) have also been isolated and sequenced (Muller-Rober et al., 1990; Nakata et al., 1991).

As Hannah and Nelson (Hannah and Nelson, 1975 and 1976) postulated, both Shrunken-2 (Sh2) (Bhave et al., 1990) and Brittle-2 (Bt2) (Bae et al., 1990) are structural genes of maize endosperm ADP-glucose pyrophosphorylase. Sh2 and Bt2 encode the large subunit and small subunit of the enzyme, respectively. From cDNA sequencing, Sh2 and Bt2 proteins have predicted molecular weight of 57,179 Da (Shaw and Hannah, 1992) and 52,224 Da, respectively. The
endosperm is the site of most starch deposition during kernel development in maize. Sh2 and bt2 maize endosperm mutants have greatly reduced starch levels corresponding to deficient levels of AGP activity. Mutations of either gene have been shown to reduce AGP activity by about 95% (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). Furthermore, it has been observed that enzymatic activities increase with the dosage of functional wild type Sh2 and Bt2 alleles, whereas mutant enzymes have altered kinetic properties. AGP is the rate limiting step in starch biosynthesis in plants. Stark et al. placed a mutant form of E. coli AGP in potato tuber and obtained a 35% increase in starch content (Stark, 1992).

The cloning and characterization of the genes encoding the AGP enzyme subunits have been reported for various plants. These include Sh2 cDNA (Bhave et al., 1990), Sh2 genomic DNA (Shaw and Hannah, 1992), and Bt2 cDNA (Bae et al., 1990) from maize; small subunit cDNA (Anderson et al., 1989) and genomic DNA (Anderson et al., 1991) from rice; and small and large subunit cDNAs from spinach leaf (Morell et al., 1987) and potato tuber (Muller-Rober et al., 1990; Nakata et al., 1991). In addition, cDNA clones have been isolated from wheat endosperm and leaf tissue (Olive et al., 1989) and Arabidopsis thaliana leaf (Lin et al., 1988).

AGP functions as an allosteric enzyme in all tissues and organisms investigated to date. The allosteric properties of AGP were first shown to be important in E. coli. A glycogen-overproducing E. coli mutant was isolated and the mutation mapped to the structural gene for AGP, designated as glyC. The mutant E. coli, known as glyC-16, was shown to be more sensitive to the activator, fructose 1,6 bisphosphate, and less sensitive to the inhibitor, cAMP (Preiss, 1984). Although plant AGP's are also allosteric, they respond to different effector molecules than bacterial AGP's. In plants, 3-phosphoglyceric acid (3-PGA) functions as an activator while phosphate (PO4) serves as an inhibitor (Dickinson and Preiss, 1969).

In view of the fact that endosperm starch content comprises approximately 70% of the dry weight of the seed, alterations in starch biosynthesis correlate with seed weight. Unfortunately, the undesirable effect associated with such alterations has been an increase in the relative starch content of the seed. Therefore, the development of a method for increasing seed weight in plants without increasing the relative starch content of the seed is an object of the subject invention.

**Brief Summary of the Invention**

The subject invention concerns a novel variant of the Shrunken-2 (Sh2) gene from maize. The Sh2 gene encodes ADP-glucose pyrophosphorylase (AGP), an important enzyme involved in starch synthesis in the major part of the corn seed, the endosperm. In a preferred embodiment, the novel gene of the subject invention encodes a variant AGP protein which has two additional amino
acids inserted into the sequence. The variant gene described herein has been termed the \textit{Sh2-m1Rev6} gene. Surprisingly, the presence of the \textit{Sh2-m1Rev6} gene in a corn plant results in a substantial increase in corn seed weight when compared to wild type seed weight, but does so in the absence of an increase in the relative starch content of the kernel.

The subject invention further concerns a method of using the variant \textit{sh2} gene in maize to increase seed weight. The subject invention also concerns plants having the variant \textit{sh2} gene and expressing the mutant protein in the seed endosperm.

As described herein, the \textit{sh2} variant, \textit{Sh2-m1Rev6}, can be produced using \textit{in vivo}, site-specific mutagenesis. A transposable element was used to create a series of mutations in the sequence of the gene that encodes the enzyme. As a result, the \textit{Sh2-m1Rev6} gene encodes an additional amino acid pair within or close to the allosteric binding site of the protein.

**Brief Description of the Sequences**

SEQ ID NO. 1 is the genomic nucleotide sequence of the \textit{Sh2-m1Rev6} gene.

SEQ ID NO. 2 is the nucleotide sequence of the \textit{Sh2-m1Rev6} cDNA.

SEQ ID NO. 3 is the amino acid sequence of the protein encoded by nucleotides 87 through 1640 of SEQ ID NO. 2.

SEQ ID NO. 4 is a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO. 5.

SEQ ID NO. 5 is the amino acid sequence of an ADP-glucose pyrophosphorylase (AGP) enzyme subunit containing a single serine insertion.

**Detailed Disclosure of the Invention**

The subject invention provides novel variants of the \textit{Shrunken-2 (Sh2)} gene and a method for increasing seed weight in a plant through the expression of the variant \textit{sh2} gene. The \textit{Sh2} gene encodes a subunit of the enzyme ADP-glucose pyrophosphorylase (AGP) in maize endosperm. One variant gene, denoted herein as \textit{Sh2-m1Rev6}, contains an insertion mutation that encodes an additional tyrosine:serine or serine:tyrosine amino acid pair that is not present in the wild type protein. The sequences of the wild type DNA and protein are disclosed in Shaw and Hannah, 1992.

The \textit{in vivo}, site-specific mutation which resulted in the tyrosine:serine or serine:tyrosine insertion, was generated in \textit{Sh2} using the transposable element, \textit{dissociation (Ds)}, which can insert into, and be excised from, the \textit{Sh2} gene under appropriate conditions. \textit{Ds} excision can alter gene expression through the addition of nucleotides to a gene at the site of excision of the element.
In a preferred embodiment, insertion mutations in the Sh2 gene were obtained by screening for germinal revertants after excision of the Ds transposon from the gene. The revertants were generated by self-pollination of a stock containing the Ds-Sh2 mutant allele, the Activator (Ac) element of this transposable element system, and appropriate outside markers. The Ds element can transpose when the Ac element is present. Wild type seed were selected, planted, self-pollinated and crossed onto a tester stock. Results from this test cross were used to remove wild type alleles due to pollen contamination. Seeds homozygous for each revertant allele were obtained from the self-progeny. Forty-four germinal revertants of the Ds-induced sh2 mutant were collected.

Cloning and sequencing of the Ds insertion site showed that the nucleotide insertion resides in the area of the gene that encodes the binding site for the AGP activator, 3-PGA (Morrell, 1988). Of the 44 germinal revertants obtained, 28 were sequenced. The sequenced revertants defined 5 isoalleles of sh2: 13 restored the wild type sequence, 11 resulted in the insertion of the amino acid tyrosine, two contained an additional serine (inserted between amino acid residues 494 and 495, respectively, of the native protein sequence), one revertant contained a two amino acid insertion, tyrosine:tyrosine, and the last one, designated as Sh2-m1Rev6, contained the two amino acid insertion, tyrosine:serine or serine:tyrosine. The Sh2-m1Rev6 variant encodes an AGP enzyme subunit that has either the serine:tyrosine amino acid pair inserted between the glycine and tyrosine at amino acid residues 494 and 495, respectively, of the native protein, or the serine:tyrosine amino acid pair inserted between the two tyrosine residues located at position 495 and 496 of the native protein sequence. Due to the sequence of the amino acids in the area of the insertions, the Sh2-m1Rev6 variant amino acid sequences encoded by each of these insertions are identical to each other.

Surprisingly, the expression of the Sh2-m1Rev6 gene in maize resulted in a significant increase in seed weight over that obtained from maize expressing the wild-type Sh2 allele. Moreover, seeds from plants having the Sh2-m1Rev6 gene contained approximately the same percentage starch content relative to any of the other revertants generated. In a preferred embodiment, the Sh2-m1Rev6 gene is contained in homozygous form within the genome of a plant seed.

The subject invention further concerns a plant that has the Sh2-m1Rev6 gene incorporated into its genome. Other alleles disclosed herein can also be incorporated into a plant genome. In a preferred embodiment, the plant is a monocotyledonous species. More preferably, the plant may be Zea mays. Plants having the Sh2-m1Rev6 gene can be grown from seeds that have the gene in their genome. In addition, techniques for transforming plants with a gene are known in the art.

Because of the degeneracy of the genetic code, a variety of different polymucleotide sequences can encode the variant AGP polypeptide disclosed herein. In addition, it is well within
the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially the same, polypeptide of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not materially alter the functional activity of the polypeptide encoded by Sh2-m1Rev6 or the other alleles. The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the wild type Sh2 DNA sequence so as to permit hybridization with that sequence under standard high-stringency conditions. Such hybridization conditions are conventional in the art (see, e.g., Maniatis et al., 1989).

The polynucleotide molecules of the subject invention can be used to transform plants to express the Sh2-m1Rev6 allele, or other alleles of the subject invention, in those plants. In addition, the polynucleotides of the subject invention can be used to express the recombinant variant AGP enzyme. They can also be used as a probe to detect related enzymes. The polynucleotides can also be used as DNA sizing standards.

The polypeptides encoded by the polynucleotides of the subject invention can be used to catalyze the conversion of ATP and α-glucose-1-phosphate to ADP-glucose and pyrophosphate, or to raise an immunogenic response to the AGP enzymes and variants thereof. They can also be used as molecular weight standards, or as an inert protein in an assay.

The following are examples which illustrate procedures and processes, including the best mode, for practicing the invention. These examples should not be construed as limiting, and are not intended to be a delineation of all possible modifications to the technique. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Expression of Sh2-m1Rev6 Gene in Maize Endosperm.

Homozygous plants of each revertant obtained after excision of the Ds transposon were crossed onto the F1 hybrid corn, "Florida Stay Sweet." This sweet corn contains a null allele for the Sh2 gene, termed sh2-R. Resulting endosperms contained one dose of the functional allele from a revertant and two female-derived null alleles, denoted by the following genotype Sh2-m1RevX/sh2-R/sh2-R, where X represents one of the various isoalleles of the revertants. Crosses were made during two growing seasons.
Resulting seed weight data for each revertant and wild type seed are shown in Table 1. The first column shows the amino acid insertion in the AGP enzyme obtained after the in vivo, site-specific mutagenesis.

<table>
<thead>
<tr>
<th>Sequence alteration</th>
<th># of revertants</th>
<th>Average Seed weight</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>13</td>
<td>0.250 grams</td>
<td>0.015</td>
</tr>
<tr>
<td>tyrosine</td>
<td>11</td>
<td>0.238 grams</td>
<td>0.025</td>
</tr>
<tr>
<td>serine</td>
<td>2</td>
<td>0.261 grams</td>
<td>0.014</td>
</tr>
<tr>
<td>tyr, tyr</td>
<td>1</td>
<td>0.223 grams</td>
<td>nd*</td>
</tr>
<tr>
<td>tyr, ser (Rev6)</td>
<td>1</td>
<td>0.289 grams</td>
<td>0.022</td>
</tr>
</tbody>
</table>

*nd = not determined

The data shown in Table 1 represents the average kernel seed weight for each revertant over the course of two growing seasons. The expression of the Sh2-m1Rev6 gene to produce the Rev6 mutant AGP subunit gave rise to an almost 16% increase in seed weight in comparison to the wild type revertant. The revertants having the single serine insertion also showed an increase in average seed weight over wild type seed weight.

In addition, starch content was determined on the kernels analyzed above using various methodologies. The analysis showed that Sh2-m1Rev6 containing kernels were no higher in percentage starch relative to kernels expressing the other alleles shown in the table above. Therefore, it appears that the increase in seed weight is not solely a function of starch content.

Corn seeds that contain at least one functional Sh2-m1Rev6 allele (the tyrosine, serine insertion) have been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 USA, on May 20, 1996 and assigned ATCC accession number ATCC 97624. Seeds having at least one functional Sh2-m1Rev20 allele (serine insertion) have also been deposited with ATCC on May 20, 1996 and assigned ATCC accession number ATCC 97625.

The seeds have been deposited under conditions that assure that access to the biological material will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposit will be available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood
that the availability of a deposit does not constitute a license to practice the subject invention in
derogation of patent rights granted by governmental action.

Further, the subject seed deposit will be stored and made available to the public in accord
with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored
with all the care necessary to keep it viable and uncontaminated for a period of at least five years
after the most recent request for the furnishing of a sample of the deposit, and in any case, for a
period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent
which may issue disclosing the seed. The depositor acknowledges the duty to replace the deposit
should the depository be unable to furnish a sample when requested, due to the condition of the
deposit. All restrictions on the availability to the public of the subject seed deposit will be
irrevocably removed upon the granting of a patent disclosing it.

As would be apparent to a person of ordinary skill in the art, seeds and plants that are
homozygous for the Sh2-m1Rev6 or the Sh2-m1Rev20 allele can be readily prepared from
heterozygous seeds using techniques that are standard in the art. In addition, the Sh2-m1Rev6 and
Sh2-m1Rev20 genes can be readily obtained from the deposited seeds.

The skilled artisan, using standard techniques known in the art, can also prepare
polynucleotide molecules that encode additional amino acid residues, such as scrine, at the location
of the insertions in the subject revertants. Such polynucleotide molecules are included within the
scope of the subject invention.

It should be understood that the examples and embodiments described herein are for
illustrative purposes only and that various modifications or changes in light thereof will be suggested
to persons skilled in the art and are to be included within the scope and purview of this application
and the scope of the appended claims.
References


NOT FURNISHED UPON FILING
(A) LENGTH: 7745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TAAGAGGGGT GCACCTAGCA TAGATTTTTT GGGCTCCCTG GCCTCTCCTT TCTTCCGCTT 60
GAAAAACAACC TACATGGATA CATCTGCAAC CAGAGGAGGT ATCTGATGCT TTTTCTGGGG 120
CAGGAGAGGC TATGAGACGT ATGTCTCTAA AGCCACTTGT CATTGTGGGA AACAAATATC 180
GATCTTTGTTC ACTTCCATCAT GCATGACAT TTGTGGAAC TACTAGCTTA CAACCATTTG 240
TGACAGCTCA GAAAAGATTT ATCTCTGAAA GATTTCAATGT GTACCCGTGGG AAATGAGAAA 300
TGTTGCAACAC TCAAAAACCT TCAATATGTT GTTGCCAGGC AAACCTCTCTT GGAAGAAGGG 360
TGTCCTAAAAC TATGAACGGG TATGAAGAAG GTAAAACAAC CCGCTTGGCA TTTTGAAGGT 420
ATCATCTATA GATGCTCTGT GAGGGGAAAG CCCTACGCCA ACGTTATTTA CTGCAAACCA 480
GCTTCAACAC ACAGTTGCTC GCTTTATGAT GCATCTCACA CCCAGGACCC CACCATCACC 540
TATTCACCTA TCTTCGCGTC CTGTTTATTTT TCTTCGCCCT TCTGATCATA AAAATCATTT 600
AAGAGTTTGC AAACATGCAT AGGCATATCA ATATGCTCAT TTATTAATTT CTTAGCAGAT 660
CATTCTCTCTA CTCTTTACTT TATTTATGTT TTGAATAATA TGTCCCTGAC CTTAGGAGCT 720
CGTATACAGT ACCAATGCACT CTTCATTTAA GTGAATTTTC AGAAAGGAG TAGGAACCTA 780
TGAGATAATT TTTCAAATTT AATTTAGGCC TCTTATTATG TTTATGACA AGGCGAAGGG 840
CAAAATCGGA ACACTAATGA TGTTGGTTG CATGAGTCTG TGATTATCCT GCAAGAAATTG 900
TGAACCCTTG TTTCTGTCGG TGAGCATATA ACAAACAGCT TCTAGGCTCT TTTAATGATAC 960
TTGCACTTGC AAGAAATGTC AAACCTCTTTT CATTCTGTA TTGGGACAATA ATGCCAAAGC 1020
ATCCAGGCTT TTTCATGGTT GTGAATCTGT TATACAGTT CATCTTCAAG CATATGCCTCT 1080
CCTCATACCT TATATAAAAAC CATGAAACGC ATCGCAATTG GCCAAAGAGT CACTTGCGGA 1140
GGCAAGTGTG ATTTGGACCT TGACGCCACC TTTTTTGTTT CGTTGTAAG TATAATTTCC 1200
CTTACCATCT TTATCTGTTA GTTTATTTTG TAATGGGAAG GATATTGTGG AAGAGGATG 1260
AGATGCTATC ATCTATGAC TCTGCAATATG CATGTGACGT TATATGGCCT GCTCCATA 1320
ATTTGAATTG CTCCATATCTT GCGCAACTA TACTGCAAGG TATATGCTAA GTTCATCA 1380
AAGTTCTGTT TTTTCAATTCT AAAAAAGCTTT TAGTTGCACG CAAATTTGTC CATGAGGAA 1440
AGGAAATCTG TTTTGTTTAC TTGCTTGAG GTCATTTTCT CATAATGCCA GTTTTATGA 1500
AGTAATAAAC TCTAGTTTGC TCTAAATATG TCAATTAAAA GGGCAAACTAT ATATTCATTG 1560
TTCAATTTAT CGTAATAAGTT CCCCCTTTTG AAAAAATAGT GCATCTATTAT ATTTGAGTTG 1620
CAGGATCTAC TAGATTGTTG AAGGATATGG CAGTTTCAC CGGCAATGGA CACGAACTCA 1680
GGTCCTCACC AGATAAGATC TTGTTAGGAG GATGGATAGG ACAGTTTGGA AAAATATTAGT 1740
ATGGGGGCGA GAAACGAGGA GAAAGCTTTG AAGAATAGTT GCTTTTGTTG TAGTTTGCT 1800
GCAAATCACA AAGTTATCTC TACCTCAGAT GCTGGTCTCG AAATCTTTTG AAGTATCCAC 1860
CTCAATTATT ACTCTTACAT GTGGTGTGG TTTACGTGG TCTTTTCAG GGAATTTAC 1920
TGATTTTTTT GTTTTTTTTG GGAGATTCTAT ACTCTGTGTT GACTGTTTAT TGTAAAGATT 1980
TGTTCAAATA GGTCATCTCA ATAATTTGTT GAAATCTGGG AACTGTGTT TCACCTGGTT 2040
CAGGAAAGA TAGATTATGG GTTTACGCTA GATAAATCTTAGA CTTTATGGGT 2100
GCTGCAATAG ATACCAAAAT CATTGCTACG ATATCCTATT AGTAAATTCA GCAGCCTGCA 2160
TTACATATAT AACTGCAACT CCTAGTTGCG TTAAAAAAA AAAATGCAAC TCTTAAAGCG 2220
CTCACAGTGC TAATCTTTCC GAAATGTTTA TTTAATGGCA TGTATGCAC ACTTGTAATAC 2280
TTATCAGAGA TTAGGAAATC TAACTCTAGG CCCCATATTG GACAGATTCTC CAAACACGT 2340
CCTCTAGGAA AAAAAATGCT GATGCAAACC GTGTATCTGC TACATTTTGC GCGGGAGGCA 2400
CTGGATCTCA GCCTTCTCTC GTCAAAAGCA CHAGAGTCTG GCCGTGCTGA AGGGATAACA 2460
CTGAACTCAC AACGTTGATT ACTCTATTAT AGTATTATAC AGACTGTACT TTTGGATT 2520
AZCTTAGTTT TCTCAAATAT TTAGGTTGCT TCTCTCATTAC TCAAGATACA CAATTGATCC 2580
ATAATCGAGAG TGGTATGAAA GACAGTGGAT TAAAAGATTA TATTTTGTGG GAGACTTCCA 2640
GTCATAATTG CCTTAGAGTTT TTTTGCGGCC AAGATTTCTG CTTTCATCTCT 2700
TTTTTAAATT TTTTAAATTG TGCAACTATTA GGGTACCTTT GAGAAGATTT ACAGGCTTAT 2760
TGATATCTCT ATGACTAAACT GCTTCAACAG TGTTATAAAT AAGATATTGT TGATGGTCA 2820
GTTCAATTCT ACTTGCGCTTA ACCGCCATAT TCATCGTACA TACCTTTGAG GCAGGATCAA 2880
CTTTGATGAT GGATCTTGAT AGGTGATTGA CTCTACCTTT TTGATGGTGA ATACCTGTAAT 2940
TAGGATGAGA TTTGCTGAGA GAGAATAATA AAGCAGTAGC GAGATCTTTT TTCAAAAGGT 3000
TAGATCAGAAA GGCAATTTGG TTTAAAAACAC TACGGACTTC TACCATTTAT GTCATTACTT 3060
(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 1919 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACAAGATCAC TTGGGAGGC AAGTGGCATTT TGATCTTGCG AGCCACCTTT TTTTGTTCTG 60
TTGGTATCT ATGATGTTAGA GGAGATATGC AGTGTGCTCT TGCAATGTGAC AGCAACTCAG 120
GTCCTCACA GATAAGATCT TGGAGGCTTGA ATGGATTTGA CAGGCTGGAA AAATATAGTA 180
TTGGGCGGCA AAGGAGGGAG AAAGCTTGGGA GAAATAGTTT CTTGCTGTTGTT AGAATGTGC 240
CAACTACACA ATGTATTTCTT ACCCTGATGCT TTGGCTGCTGA AACTCTTCTA TCTCAACAC 300
AGTCCTCTTAG GAAAAAAATTT GCTGATGCAC ACCGCTCATC TGCTGACATT TGGGGGCGG 360
GCACCTGGATC TCAGCTTTTT CCTCTGACAA GCACAGAGAC TAGGCCTGGT GTACCTGTGG 420
GAGATGTTGCA GGGTCTATTT GATATCCTCTA TCGAGAATCT GTCCAAACGT GTGATTAAA 480
AGATTATTTGGT GATGATGCAG TTCAATTTCTA GCTAGGCTTAA CCAGCTATAT CATCGTACAT 540
ACCTGGAACCG CGGATCACAC TTGGCTGTAGT GACTCTGATC GGTTATAGCG GCATACAAAA 600
TCGCCTGAAGG CCAAGCTTGG TGGTTCAGGG ATGACGCAAG CTTACATTAG AAATTTATTCTCT 660
GGTTACTTGGA GAGTTATTAG ATGCACCAAA CCTGACCAAA CATGTGACAT TTGAGTGGCC 720
ATCATGTTTAT TCGGATGAAT CACATGGAAC TTGTGCAAGG AACTGTCGAG GACGATGCCT 780
ATATCAGCTAT ATCATGCTTGT CCTGTTGATG AGAGCCGAGT TCTAAATATT GGGCTAGTGA 840
AGATGATCAG TACTGGACGG GTACTCTTAT TTGTGGAAAT ACCAAAGGGT GCTGATTGTGGA 900
ATTCTATGACG AGTTGAGACA AACTTCTCTGA GCTATGCTAT AGATGATGCA CAGAAATATCT 960
CATACCTTGCC ATCAATGGGC ATTTATGCTT TCAGAAAATA TGCACTTTTA GACCTTTCTCA 1020
AGTCAAAAATA TACTCAATTG CATGACTTTTGT GATCTGGAAT CCTCCCAAGA GCTGTACTAG 1080
ATCATGTTGT GCAGGCTATG ATTTTTAGGG GCTATTTGGGA GGATTTGGA ACAATCAAAAT 1140
CATATTCTTG GACCCCAACTTG GCTACTCTTT CATAGGTGAGTTTCTTCTC 1200
CARACACCCAC TTCTCTTACT GCACCCGGAT GCTGCGTCTC GACCGAAATT GACAAATGCA 1260
AGATGAAATA TGCAATTTATC TCAGATGGTT GCTTACTGAG AGAAATGCAA ATCGGGCAAA 1320
CTTGTATGGG ATCGCTCTCA GCTGTCAGCT CTGGAGTGTG ACTCAAAGGAC TCCGGATGTA 1380
TGGGACGGCA GAATCATGAA ACTGAAAGAG AAGCTTCAAA GCTACTGTTA GCTGGGAAAG 1440
TCCCGATGTTG AATAGGAAAG AACAAAGAAG TTAGGAACTG TATCATGAC TGGATATAGTA 1500
GGATTGGAGAA GACGGGCTAG ATACAAACAA TGGAGGGCAT CAAAGGGCT GATCACCCGG 1560
AAGAAGGGTA CTGGTACTAC ATAAGGTTGGT GAATCGTGTG ATCTCGAAG AATGCAAACCA 1620
TCAACGATGG GTCTGTCATA TAGATCGGCT GCGTTGGCT CTCACAAAACA AGAACCTACA
ATGGTATTCG ATCGATGGAT CGTGTAACCT TGGTATGGTA AGACCCGCTT GACAGGAAGT
CGAGCTTCCG GCCAAGATGC TAGTCTGGCA TGCTGGTCTTGACCAATTGGTCTAGT
ATGTACCTGT TATAAGCTGC CTTAGAAGTT GCAAGCAAACC TTTTTATGAA CTTTGTATT
TCCATTACCC TGCTTTGGAT CAACCTATATC TGTCAGTCCT ATATAATCT AAATTTTTA

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 518 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met  Gln  Phe  Ala  Leu  Ala  Leu  Asp  Thr  Asn  Ser  Gly  Pro  His  Gln  Ile
  1    5   10    15
Arg  Ser  Cys  Gly  Asp  Gly  Asp  Gly  Ile  Asp  Arg  Leu  Glu  Lys  Leu  Ser  Ile
  20   25   30
Gly  Gly  Arg  Lys  Gln  Glu  Lys  Ala  Leu  Arg  Asn  Arg  Cys  Phe  Gly  Gly
  35   40   45
Arg  Val  Ala  Ala  Thr  Thr  Gln  Cys  Ile  Leu  Thr  Ser  Asp  Ala  Cys  Pro
  50   55   60
Glu  Thr  Leu  His  Ser  Gln  Thr  Gln  Ser  Ser  Arg  Lys  Asn  Tyr  Ala  Asp
  65   70   75   80
Ala  Asn  Arg  Val  Ser  Ala  Ile  Leu  Gly  Gly  Thr  Gly  Ser  Gln
  85   90   95
Leu  Phe  Pro  Leu  Thr  Ser  Thr  Arg  Ala  Thr  Pro  Ala  Val  Pro  Val  Gly
 100  105  110
Gly  Cys  Tyr  Arg  Leu  Ile  Asp  Ile  Pro  Met  Ser  Asn  Cys  Phe  Asn  Ser
 115  120  125
Gly  Ile  Asn  Lys  Ile  Phe  Val  Met  Ser  Gln  Phe  Asn  Ser  Thr  Ser  Leu
 130  135  140
Asn  Arg  His  Ile  His  Arg  Thr  Tyr  Leu  Glu  Gly  Ile  Asn  Phe  Ala
 145  150  155  160
Asp  Gly  Ser  Val  Gln  Val  Leu  Ala  Ala  Thr  Gln  Met  Pro  Glu  Glu  Pro
 165  170  175
<table>
<thead>
<tr>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala Gly Trp Phe Glu Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp</td>
</tr>
<tr>
<td>180</td>
</tr>
<tr>
<td>Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile</td>
</tr>
<tr>
<td>195</td>
</tr>
<tr>
<td>Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln</td>
</tr>
<tr>
<td>210</td>
</tr>
<tr>
<td>Lys His Val Glu Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val</td>
</tr>
<tr>
<td>225</td>
</tr>
<tr>
<td>Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr</td>
</tr>
<tr>
<td>245</td>
</tr>
<tr>
<td>Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn</td>
</tr>
<tr>
<td>260</td>
</tr>
<tr>
<td>Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala</td>
</tr>
<tr>
<td>275</td>
</tr>
<tr>
<td>Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys</td>
</tr>
<tr>
<td>290</td>
</tr>
<tr>
<td>Asp Ala Leu Leu Asp Leu Lys Ser Lys Tyr Thr Gln Leu His Asp</td>
</tr>
<tr>
<td>305</td>
</tr>
<tr>
<td>Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln</td>
</tr>
<tr>
<td>325</td>
</tr>
<tr>
<td>Ala Cys Ile Phe Thr Gly Tyr Trp Glu Asp Val Gyl Thr Ile Lys Ser</td>
</tr>
<tr>
<td>340</td>
</tr>
<tr>
<td>Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp</td>
</tr>
<tr>
<td>355</td>
</tr>
<tr>
<td>Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro</td>
</tr>
<tr>
<td>370</td>
</tr>
<tr>
<td>Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp</td>
</tr>
<tr>
<td>385</td>
</tr>
<tr>
<td>Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val</td>
</tr>
<tr>
<td>405</td>
</tr>
<tr>
<td>Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met</td>
</tr>
<tr>
<td>420</td>
</tr>
<tr>
<td>Gly Ala Asp Ile Tyr Glu Thr Glu Glu Ala Ser Lys Leu Leu Leu</td>
</tr>
<tr>
<td>435</td>
</tr>
<tr>
<td>Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn</td>
</tr>
<tr>
<td>450</td>
</tr>
<tr>
<td>Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr</td>
</tr>
<tr>
<td>465</td>
</tr>
</tbody>
</table>
Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Tyr Ser
\[ \begin{array}{c}
485 \\
490 \\
495 \\
\end{array} \]

Tyr Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile
\[ \begin{array}{c}
500 \\
505 \\
510 \\
\end{array} \]

Asn Asp Gly Ser Val Ile
\[ \begin{array}{c}
515 \\
\end{array} \]

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1551 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATGCAGTTTTG CACTTGCACT GGACAGCAGC TCAGGTCTTC ACCAGATAAG ATCTTGTGAG 60
GGTGATGAGG TTGAGAGTTT GGAATTGATAGT GGAAGCGCA GAGGAAGCT
120
TGGAAAGGAA TGGGCTTGGG GTGATAGTG CCTGCAAAGT TCTTACCTCA 180
GATGCTTGTC CTGAAACTCT CTATTTCACA ACACAAGCTCT CTAGGAAAAA TTATGCCTGAT
240
GCAAACCGTG TATCTCGGAT CATTCTGGCC GAGCAGACTG TATCTAGCTGT 300
ACAGCAGCAG GAGCTAGGCA TGGCTGACCT GTGGAGGAGT TTTCAGGGCT CATTATAGTC
360
CCTATGAGTA ACTGCTTCAA CAGTGTATA AATAAGATAT TTGTGATGAG TCAGGGTCAAT
420
TCTACTTTCGC TTAACCAGCCA TATTCATCGT ACATAATCTTG AAGGCGGGAT CAACTTTGCT
480
GATGGATCTG TACAGTTATT AAGCGGCTACA CAAATGCTTG AAGGACACGC TGGATGTTCC
540
CAGGTACAG CAGACTCTAT CAGGAATATT ATCTGGGTAC TGGAGATTT TACACGCAC
600
AAATCCATTG ACAACATTGT AATCTTGGAT GGGAGCACG TTTATCGGAA GAATTACATG
660
GAACCTGTGC AGAACACGTG CGAGGACCAG GCTGATATCA CTAATATATG TGCTCTCTTT
720
GATGAGGAGG GAGCTCTCA AAATGCGCTA TGGAAGATTT ATCGATCGGC ACGTGTACCT
780
CAATTCCTTG AAAAAACCAA GGGTGCTGAT TTGAACTTCA TGAGCGTCCG GACCAACTTC
840
CTGAGCTATG CTATGATGAA TGCAAGAAA TAATCCTACG TTGCACTATG GGGATTATATT
900
GTCTCATGAA AAGATGCACG TTATGACCT TTCAAGTCAA AAATATACAT TATACATGAC
960
TTTGGATCTG AAATCCCTCC AAGGACTGTG CATGATCATG GGGAGCGGC ATGGGATTTT
1020
ACGGGCTATT GGGGAGATGT TGGAAACACT CAAATCATTCT TTGTGACTCA CTGGGCCCTC
1080
ACTGAGCAGC CTCCAAGTT TGATTTTAC GATCCAAAAA CACCTTTCTT CACTGCACCC
CGATGCTTGC CTCGCAGGCA ATTGGACAAG TGCAAGATGA AATATGCATT TATCTCAGAT
GGTTGCTTAC TGAGAGAATG CAACATCGAG CATTCTGTGA TTGGAGCTTG CTCACGTCGTC
AGCTCTGGAT GTGAACTCAA GGACTGCGTG ATGATGGGAG CGGACATCTA TGAAAAGCTGA
GAAGAGCTTT CCAAGCTCTT GTTAGCTGGG AAGGTCCCAGA TTGGGAATAGG AAGGAACACA
AAGATAAGGA ACTGTATCAT TGACATGAAT GCTAGGATTG GGAAGAACGT GGTGATCACA
AACAGTAAGG GCAATCCAAGA GGCCTGACAC CCGGAAGAAG GGTCCCTACTA CATAGGTCTT
GGAATCGTGTT TGATCTGCAAA GAATGCAACC ATCAACAGATG GGTCTGTCAT A

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

1  Met  Gln  Phe  Ala  Leu  Ala  Leu  Asp  Thr  Asn  Ser  Gly  Pro  His  Gln  Ile
5
10
 Arg  Ser  Cys  Glu  Gly  Asp  Gly  Ile  Asp  Arg  Leu  Glu  Lys  Leu  Ser  Ile
20
25
30
 Gly  Gly  Arg  Lys  Glu  Gly  Ala  Leu  Arg  Asn  Arg  Cys  Phe  Gly  Gly
35
40
45
 Arg  Val  Ala  Ala  Thr  Thr  Gln  Cys  Ile  Leu  Thr  Ser  Asp  Ala  Cys  Pro
50
55
60
 Glu  Thr  Leu  His  Ser  Gln  Thr  Gln  Ser  Asp  Lys  Asn  Tyr  Ala  Asp
65
70
75
 Ala  Asn  Arg  Val  Ser  Ala  Ile  Leu  Gly  Gly  Thr  Gly  Ser  Gln
85
90
95
 Leu  Phe  Pro  Leu  Thr  Ser  Thr  Arg  Ala  Thr  Pro  Ala  Val  Pro  Val  Gly
100
105
110
 Gly  Cys  Tyr  Arg  Leu  Ile  Asp  Ile  Pro  Met  Ser  Asn  Cys  Phe  Asn  Ser
115
120
125
 Gly  Ile  Asn  Lys  Ile  Phe  Val  Met  Ser  Gln  Phe  Asn  Ser  Thr  Ser  Leu
130
135
140
21

Asn Arg His Ile His Arg Thr Tyr Leu Glu Gly Gly Ile Asn Phe Ala 145 150 155 160

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

Ala Gly Trp Phe Gln Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp 180 185 190

Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile 195 200 205

Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln 210 215 220

Lys His Val Glu Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val 225 230 235 240

Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr 245 250 255

Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn 260 265 270

Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala 275 280 285

Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys 290 295 300

Asp Ala Leu Leu Asp Leu Leu Lys Ser Lys Tyr Thr Gln Leu His Asp 305 310 315 320

Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln 325 330 335

Ala Cys Ile Phe Thr Gly Tyr Trp Glu Val Gly Thr Ile Lys Ser 340 345 350

Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp 355 360 365

Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro 370 375 380

Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp 385 390 395 400

Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val 405 410 415

Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met 420 425 430

Gly Ala Asp Ile Tyr Glu Thr Glu Glu Ala Ser Lys Leu Leu Leu 435 440 445
Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn
450 455 460

Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr
465 470 475 480

Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Ser Tyr
485 490 495

Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile Asn
500 505 510

Asp Gly Ser Val Ile
515
Claims

1. A polynucleotide molecule, comprising a variant of the wild type \textit{shrunk-2} (\textit{Sh}2) gene, wherein said variant codes for the insertion of at least one additional amino acid within or close to the allosteric binding site of the ADP-glucose pyrophosphorylase (AGP) enzyme subunit, whereby a plant expressing said polynucleotide molecule has increased seed weight relative to the seed weight of a plant expressing the wild type \textit{Sh}2 gene.

2. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes at least one serine residue inserted between amino acids 494 and 495 of the native AGP enzyme subunit.

3. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes the amino acid pair tyrosine:serine, wherein said amino acid pair is inserted between amino acids 494 and 495 of the native AGP enzyme subunit.

4. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes the amino acid pair serine:tyrosine, wherein said amino acid pair is inserted between amino acids 495 and 496 of the native AGP enzyme subunit.

5. The polynucleotide molecule, according to claim 1, wherein the AGP enzyme encoded by said polynucleotide molecule consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO. 5 and SEQ ID NO. 3.

6. The polynucleotide molecule, according to claim 5, wherein the nucleotide sequence encoding SEQ ID NO. 3 comprises nucleotides 87 through 1640 of the sequence shown in SEQ ID NO. 2 or a degenerate fragment thereof.

7. A method for increasing the seed weight of a plant, comprising incorporating the polynucleotide molecule of claim 1 into the genome of said plant and expressing the protein encoded by said polynucleotide molecule.

8. The method, according to claim 7, wherein said plant is \textit{Zea mays}.
9. A plant seed comprising the polynucleotide molecule of claim 1 within the genome of said seed.

10. A plant expressing the polynucleotide molecule of claim 1.

11. The plant, according to claim 10, wherein said plant is Zea mays.

12. The plant, according to claim 10, wherein said plant is grown from the seed of claim 9.

13. A variant ADP-glucose pyrophosphorylase (AGP) protein, wherein said protein has at least one additional amino acid inserted within or close to the allosteric binding site of the wild-type AGP protein.

14. The variant AGP protein, according to claim 13, wherein said protein has at least one serine residue inserted between amino acids 494 and 495 of the wild type AGP protein sequence.

15. The variant AGP protein, according to claim 11, wherein said protein has the amino acid pair tyrosine:serine inserted between amino acids 494 and 495 of the wild-type AGP protein sequence.

16. The variant AGP protein, according to claim 11, wherein said protein has the amino acid pair serine:tyrosine inserted between amino acids 495 and 496 of the wild-type AGP protein sequence.

17. The variant AGP protein, according to claim 13, wherein said protein consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO. 5 and SEQ ID NO. 3.

18. The variant AGP protein, according to claim 13, wherein said protein is expressed in the endosperm of a plant during seed development.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

| IPC  | C12N15/62 | C12N15/54 | A01H5/00 | A01H5/10 |

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

| IPC  | C12N | A01H |

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>PROC. NATL. ACAD. SCI. USA, vol. 93, no. 12, 11 June 1996, pages 5824-9, XP000652281 M.J. GIROUX ET AL.: &quot;A single gene mutation that increases maize seed weight&quot; see the whole document. ---</td>
<td>1-18</td>
</tr>
<tr>
<td>A</td>
<td>PLANT CELL, vol. 2, 1990, pages 581-8, XP000652283 M.R. BHAVE ET AL.: &quot;Identification and molecular characterization of Shrunken-2 cDNA clones of maize&quot; cited in the application see the abstract. -----</td>
<td></td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document on which the priority of another document is based
- "O" document referred to in anoral disclosure, use, exhibition or other means

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone or in combination with one or more other such documents, such combination being obvious to a person skilled in the art.
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"A" document member of the same patent family

Date of the actual completion of the International search: 9 June 1997

Date of mailing of the International search report: 20.06.97

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016

Authorized officer: Yeats, S