

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2009234464 B2**

(54) Title
Heat resistant plants and plant tissues and methods and materials for making and using same

(51) International Patent Classification(s)
C12N 15/29 (2006.01) **C07K 14/415** (2006.01)
A01H 1/00 (2006.01) **C12N 15/52** (2006.01)

(21) Application No: **2009234464** (22) Date of Filing: **2009.03.26**

(87) WIPO No: **WO09/126208**

(30) Priority Data

(31) Number	(32) Date	(33) Country
12/082,339	2008.04.09	US

(43) Publication Date: **2009.10.15**

(44) Accepted Journal Date: **2015.01.29**

(71) Applicant(s)
University of Florida Research Foundation, Inc.

(72) Inventor(s)
Hannah, L. Curtis; Georgelis, Nikolaos

(74) Agent / Attorney
Spruson & Ferguson, L 35 St Martins Tower 31 Market St, Sydney, NSW, 2000

(56) Related Art
US 6969783

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 October 2009 (15.10.2009)

(10) International Publication Number
WO 2009/126208 A3

(51) International Patent Classification:

C12N 15/29 (2006.01) C07K 14/415 (2006.01)
C12N 15/52 (2006.01) A01H 1/00 (2006.01)

(21) International Application Number:

PCT/US2009/001903

(22) International Filing Date:

26 March 2009 (26.03.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

12/082,339 9 April 2008 (09.04.2008) US

(71) Applicant (for all designated States except US): **UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.** [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HANNAH, L., Curtis** [US/US]; 4400 N.W. 39th Avenue, Apt. 434, Gainesville, FL 32606 (US). **GEORGELIS, Nikolaos** [GR/US]; 1241 S.W. 4th Avenue, Apt. 22, Gainesville, FL 32601-6311 (US).

(74) Agents: **PACE, Doran, R.** et al.; Saliwanchik, Lloyd & Saliwanchik, Po Box 142950, Gainesville, FL 32614-2950 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

18 March 2010

(54) Title: HEAT RESISTANT PLANTS AND PLANT TISSUES AND METHODS AND MATERIALS FOR MAKING AND USING SAME

(57) Abstract: The subject invention concerns materials and methods for providing plants or plant tissue with increased resistance to heat conditions and/or increased starch biosynthesis. Increased resistance of a plant or plant tissue to heat conditions provides for decreased yield losses as compared to the yield losses generally observed at elevated temperatures. One aspect of the invention concerns polynucleotides that encode a mutant plant small subunit of AGP ase. The subject invention also comprises a mutant plant small subunit of AGP ase encoded by a polynucleotide of the invention. The subject invention also concerns plants comprising a polynucleotide of the invention and method for making the plants.



WO 2009/126208 A3

DESCRIPTION

HEAT RESISTANT PLANTS AND PLANT TISSUES AND METHODS AND
MATERIALS FOR MAKING AND USING SAME

GOVERNMENT SUPPORT

The subject matter of this application has been supported by a research grant from the National Science Foundation under grant number IOS-0444031. Accordingly, the government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Heat stress leads to decreased maize yield (Peters *et al.*, 1971; Thompson, 1975; Chang, 1981; Christy and Williamson, 1985). This can be attributed to reduced photosynthate availability and transportation from source to sink tissues, poor pollination, reduced cell and granule size and number, early seed abortion and/or reduced grain filling period. Growth of endosperm starts with a lag phase in which cells actively divide and continues with a linear phase in which cells increase in size and starch synthesis occurs. Elevated temperature during lag phase resulted in reduced yield (Jones *et al.*, 1984). These investigators suggested that reduced yield was due to reduced cell and granule number and size as well as seed abortion. Additionally, elevated temperatures during the linear phase resulted in shorter grain filling period and subsequently smaller kernels (Jones *et al.*, 1984). Similar results were found by Hunter *et al.* (1977) and Tollenaar and Bruulsema (1988).

Records from five states that traditionally produce more than 50% of the US corn showed that average daily temperature was 23.6°C, around 2°C higher than optimum during grain filling (Singletary *et al.*, 1994). Photosynthate availability during grain filling is not reduced at high temperatures, at least in barley and wheat. Indeed, sucrose content in barley and wheat seeds was either unchanged or elevated at high temperatures (Bhullar and Jenner, 1986; Wallwork *et al.*, 1998). Also photosynthesis in maize increases up to 32°C (Duncan and Hesketh, 1968; Hofstra and Hesketh, 1969; Christy *et*

al., 1985). Moreover, Cheikh and Jones (1995) studied the ability of maize kernels to fix ^{14}C sucrose and hexoses at different temperatures. They found that these sugars increased in the seed at elevated temperatures. The evidence above suggests that limited sugar availability and transport into the kernel during grain filling are not the cause of
5 temperature-induced yield decreases.

There have been extensive efforts to identify biochemical pathways that impact grain filling during elevated temperatures. Singletary *et al.* (1993; 1994) assayed starch biosynthetic enzymes in maize kernels grown *in vitro* at elevated temperatures (22°C to 36°C). They found that ADP-glucose pyrophosphorylase (AGPase) and soluble starch
10 synthase (SSS) were more heat labile compared to other enzymes participating in starch synthesis. They suggested that heat lability of AGPase and SSS contributes to grain filling cessation. Duke and Doehlert (1996) found that transcripts of several genes encoding enzymes of the starch synthesis pathway, including those encoding AGPase, were decreased at 35°C compared to 25°C. However, enzyme assays showed that only
15 AGPase activity was strikingly lower. They suggested that this could be due to a higher turnover rate of AGPase compared to other enzymes. Finally, Wilhelm *et al.* (1999), through Q_{10} analysis, showed that AGPase had the most pronounced reduction in activity compared to several other enzymes. Maize AGPase indeed lost 96% of its activity when heated at 57°C for 5 min (Hannah *et al.*, 1980).

20 AGPase catalyzes the first committed step in starch (plants) and glycogen (bacteria) synthesis. It involves the conversion of glucose-1-P (G-1-P) and ATP to ADP-glucose and pyrophosphate (PPi). AGPase is a heterotetramer in plants consisting of two identical small and two identical large subunits. The large and the small subunits are encoded by *shrunk-2* (*Sh2*) and *brittle-2* (*Bt2*) respectively in maize endosperm.
25 AGPase is allosterically regulated by small effector molecules that are indicative of the energy status of the cell. AGPase is activated by 3-PGA, the first carbon assimilatory product, and inhibited/deactivated by inorganic phosphate (Pi) in cyanobacteria, green algae and angiosperms.

The importance of maize endosperm AGPase in starch synthesis has been shown
30 by the kernel phenotype of mutants in either subunit of the enzyme. Indeed, such mutants result in shrunken kernels and a large reduction in endosperm starch content (Tsai and Nelson, 1966; Hannah and Nelson, 1976). There is also evidence that AGPase catalyses a

rate-limiting step in starch synthesis (Stark *et al.* 1992; Giroux *et al.* 1996; Greene *et al.* 1998; Sakulsingharoja *et al.* 2004; Obana *et al.* 2006; Wang *et al.* 2007).

Greene and Hannah (1998a) isolated a mutant form of maize AGPase with a single amino acid change in the large subunit termed *HS33*. They showed that the altered enzyme was more heat-stable and that stability was due to stronger subunit-subunit interactions. When wheat and rice were transformed with a *Sh2* variant that contains the *HS33* change along with a change that affects the allosteric properties of AGPase (*Rev6*) (Giroux *et al.*, 1996), yield was increased by 38% and 23% respectively (Smidansky *et al.*, 2002; 2003). Remarkably, the increase was due to an increase in seed number rather than individual seed weight.

Transformation of maize with the *Sh2* variant containing the *Rev6* and *HS33* changes also gives rise to enhanced seed number. Seed yield/ear can be increased up to 68% in maize. A detailed characterization of the maize transgenic events is under way (Greene and Hannah, in preparation). Enhanced seed number cannot be explained by *Rev6* since, when expressed alone in maize, it increases only seed weight (Hannah, unpublished). The above studies show the importance of AGPase heat stability in cereal yield.

Cross *et al.* (2004) generated a mosaic small subunit (MP) consisting of the first 200 amino acids of BT2 and the last 275 amino acids of the potato tuber small subunit. MP in a complex with SH2 had several features that could lead to agronomic gain (Cross *et al.*, 2004; Boehlein *et al.*, 2005). Some of those features were increased activity in the absence of the activator 3-PGA, increased affinity for 3-PGA and elevated heat stability compared to wildtype maize endosperm AGPase (BT2/SH2). Preliminary data show that maize plants with transgenic MP containing AGPase variant expressed in maize endosperm provides for a starch yield increase (Hannah, unpublished data).

BRIEF SUMMARY OF THE INVENTION

The subject invention concerns materials and methods for providing plants or plant tissue with increased resistance to heat conditions and/or increased starch biosynthesis. Increased resistance of a plant or plant tissue to heat conditions provides for decreased yield losses as compared to the yield losses generally observed at elevated temperatures. One aspect of the invention concerns polynucleotides that encode a mutant

plant small subunit of AGPase. Herein disclosed is a polynucleotide that encodes a plant AGPase small subunit having an amino acid mutation wherein the threonine amino acid corresponding to amino acid position 462 of wild type maize AGPase small subunit is substituted with an amino acid that confers increased heat stability. Also herein disclosed is a polynucleotide that encodes a chimeric plant AGPase small subunit composed of sequences from two different plants (as described in U.S. Patent No. 7,173,165) and comprising an amino acid mutation of the invention wherein the threonine amino acid corresponding to amino acid position 462 of wild type maize AGPase small subunit is substituted with an amino acid that confers increased heat stability. The mutation in the chimeric AGPase synergistically enhances heat stability. Also herein disclosed is a mutant plant small subunit of AGPase encoded by a polynucleotide of the invention. Characterization of heat stability as well as kinetic and allosteric properties indicates increased starch yield is provided when the polynucleotides of the invention are expressed in plants such as monocot endosperms.

Thus, according to a first embodiment of the invention, there is provided an isolated polynucleotide encoding a mutant plant AGPase small subunit protein, or a functional fragment of said protein, said protein comprising an amino acid mutation wherein the amino acid corresponding to the threonine amino acid at position 462 of wild type maize endosperm AGPase small subunit protein is replaced by an amino acid that confers increased heat stability when said mutant AGPase small subunit is expressed to form an AGPase enzyme.

According to a second embodiment of the invention, there is provided a polynucleotide encoding a chimeric plant AGPase small subunit protein, or a functional fragment of said protein, wherein said chimeric AGPase protein comprises an N-terminus sequence from an N-terminus region of a plant AGPase small subunit from a first plant and a C-terminus sequence from a C-terminus region of a plant AGPase small subunit from a second plant, and said chimeric plant AGPase small subunit protein comprises an amino acid mutation wherein the amino acid corresponding to the threonine amino acid at position 462 of wild type maize endosperm AGPase small subunit protein is replaced by an amino acid that confers increased heat stability when said mutant AGPase small subunit is expressed to form an AGPase enzyme.

According to another embodiment of the invention, there is provided an isolated polypeptide encoded by:

- a) a polynucleotide according to the first embodiment of the invention; or
- b) a polynucleotide according to the second embodiment of the invention.

According to another embodiment of the invention, there is provided an isolated mutant plant AGPase enzyme comprising one or more mutant polypeptides of the invention.

According to another embodiment of the invention, there is provided a transformed or

transgenic plant or plant tissue or cell comprising:

- a) a polynucleotide according to the first embodiment of the invention; or
- b) a polynucleotide according to the second embodiment of the invention; or
- c) the polynucleotides of both a) and b).

According to another embodiment of the invention, there is provided a method of increasing resistance of a plant to heat stress conditions and/or increasing starch biosynthesis of a plant, said method comprising incorporating one or more polynucleotide into the genome of a plant and expressing the protein encoded by said polynucleotide, wherein said polynucleotide is or comprises:

- a) any polynucleotide according to the first embodiment of the invention; or
- b) any polynucleotide according to the second embodiment of the invention.

According to another embodiment of the invention, there is provided a method for preparing a plant having an AGPase enzyme that provides for increased heat stability and/or increased starch biosynthesis in the plant relative to a plant having wild type AGPase enzyme, said method comprising introducing one or more polynucleotide into a plant cell and growing a plant from said plant cell, wherein said polynucleotide is or comprises:

- a) any polynucleotide according to the first embodiment of the invention; or
- b) any polynucleotide according to the second embodiment of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows glycogen produced by *E. coli* cells expressing BT2, BT2-TI, MP, MP-TI along with SH2. Glycogen from cells expressing only SH2, BT2, BT2-TI, and MP alone. Glycogen is measured in glucose units. The error bars indicate standard deviation (N=3).

Figures 2 A and 2B show dot blots of crude extracts from *E. coli* cells expressing BT2, BT2-TI, MP, MP-TI with the complementary subunit SH2. AGPase was visualized by using a monoclonal antibody against BT2. The density of the spots was estimated by using ImageJ (<http://rsb.info.nih.gov/ij/>).

Figure 3 shows specific activity of AGPase variants in crude and partially purified protein extracts from non-induced *E. coli* cells. Activity was measured in the reverse direction, n.d. = not detectable. The error bars indicate standard deviation (N=3).

Figure 4 shows purification of AGPase. SDS-PAGE of purified recombinant BT2/SH2, TI/SH2, MP/SH2, and MP-TI/SH2. Precision Plus Protein All Blue Standard from Biorad was used as a marker. The upper arrow on the left points to the large subunit. The lower arrow on the left points to the small subunit.

Figures 5A and 5B show heat stability of purified BT2/SH2, BT2-TI/SH2, and MP/SH2. The half-life ($T_{1/2}$) of each AGPase is expressed as mean $\pm$ standard error. The p-values are estimated by an F-test implemented by Prism (Graph pad, San Diego CA). In Figure 5A, the assay was conducted in the forward direction. In Figure 5B, the assay was conducted in the reverse direction.

Figures 6A and 6B show heat stability of purified MP/SH2 and MP-TI/SH2. The half-life ($T_{1/2}$) of each AGPase is expressed as mean $\pm$ standard error. The p-values are estimated by an F-test implemented by Prism (Graph pad, San Diego CA). In Figure 6A, the assay was conducted in the forward direction. In Figure 6B, the assay was conducted in the reverse direction.

Figures 7A-7C show 3D modeling of BT2 and TI. Figure 7A is the predicted 3D structure of BT2 monomer. The TI change is marked by a red circle. The areas of BT2 that are directly involved in subunit-subunit interactions are highlighted by yellow boxes. Figure 7B shows the distances of carbon atoms of Thr462 (1,2) from those of Pro60 (4,5) and Leu61 (3). Figure 7C shows the distances of carbon atoms of Ile462 (1,2,3,4) from those of Pro60 (5,6) and Leu61 (7,8). The Thr462 and Ile462 contacting residues were determined by using FirstGlance Jmol. Dark gray spheres indicate carbon atoms of Thr462 and Ile462. Light gray spheres indicate carbon atoms of contacting residues. Oxygen and nitrogen atoms are indicated by red and blue color respectively.

Figure 8 show strength of AGPase subunit-subunit interactions. SH2 was used as a bait and BT2, TI, and MP as a prey in a yeast two hybrid system. A quantitative β -galactosidase assay was used to quantify the interactions between the bait and the prey. The error bars indicate 2 x standard error (N=4).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (TI) of the invention.

SEQ ID NO:2 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

SEQ ID NO:3 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (MP-TI) of the invention.

SEQ ID NO:4 is an amino acid sequence of a mutant polypeptide (MP-TI) of the invention.

SEQ ID NO:5 is an amino acid sequence of a mutant polypeptide (TI + YC) of the invention.

5 SEQ ID NO:6 is an amino acid sequence of a mutant polypeptide (TI + QTCL) of the invention.

SEQ ID NO:7 is an amino acid sequence of a mutant polypeptide (TI + ETCL) of the invention.

10 SEQ ID NO:8 is an amino acid sequence of a mutant polypeptide (MP-TI + YC) of the invention.

SEQ ID NO:9 is an amino acid sequence of a mutant polypeptide (MP-TI + QTCL) of the invention.

SEQ ID NO:10 is an amino acid sequence of a mutant polypeptide (MP-TI + ETCL) of the invention.

15 SEQ ID NO:11 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:5) of the invention.

SEQ ID NO:12 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:6) of the invention.

20 SEQ ID NO:13 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:7) of the invention.

SEQ ID NO:14 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:8) of the invention.

SEQ ID NO:15 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:9) of the invention.

25 SEQ ID NO:16 is a polynucleotide sequence comprising a nucleotide sequence encoding a mutant polypeptide (SEQ ID NO:10) of the invention.

SEQ ID NO:17 is an oligonucleotide that can be used according to the present invention.

30 SEQ ID NO:18 is an oligonucleotide that can be used according to the present invention.

SEQ ID NO:19 is an oligonucleotide that can be used according to the present invention.

SEQ ID NO:20 is an oligonucleotide that can be used according to the present invention.

SEQ ID NO:21 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

5 SEQ ID NO:22 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

SEQ ID NO:23 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

10 SEQ ID NO:24 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

SEQ ID NO:25 is an amino acid sequence of a mutant polypeptide (TI) of the invention.

SEQ ID NO:26 is an amino acid sequence of potato tuber AGPase small subunit protein.

15

DETAILED DESCRIPTION OF THE INVENTION

The subject invention concerns materials and methods for providing plants with increased resistance to heat conditions and/or increased starch biosynthesis. Increased resistance of a plant to heat conditions provides for decreased yield losses as compared to yield losses generally observed at elevated temperatures.

20 One aspect of the invention concerns polynucleotides that encode a mutant plant small subunit of AGPase. In one embodiment, a polynucleotide of the invention encodes a plant AGPase small subunit having an amino acid mutation wherein the threonine amino acid corresponding to amino acid position 462 of wild type maize endosperm AGPase small subunit is substituted with an amino acid that confers increased heat stability. In a specific embodiment, the amino acid substituted is an isoleucine. In one embodiment, the mutant plant AGPase small subunit is maize endosperm AGPase small subunit. In an exemplified embodiment, the mutant plant AGPase small subunit comprises the amino acid sequence shown in SEQ ID NO:2, or a fragment or variant thereof. In a specific embodiment, the polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:1, or a fragment or variant thereof. In another embodiment, the mutant plant AGPase small subunit is barley, wheat, sorghum, potato, or rice. In a

30

specific embodiment, the mutant barley, wheat, sorghum, or potato AGPase small subunit comprises the amino acid sequence shown in SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, respectively. In another embodiment, the polynucleotide encodes a mutant plant AGPase small subunit that can additionally
5 comprise an amino acid mutation as described in published International patent application WO 2005/019425 (Hannah and Linebarger). In one embodiment, the mutant AGPase small subunit encoded by the polynucleotide comprises an amino acid mutation wherein the tyrosine corresponding to amino acid position 36 of wild type maize endosperm AGPase is substituted with a cysteine. The mutant AGPase small subunit can
10 also optionally comprise an amino acid inserted between the serine and threonine amino acids corresponding to amino acid positions 34 and 35 of wild type maize endosperm AGPase, respectively. In specific embodiments, the amino acid inserted between amino acids at position 34 and 35 of the AGPase small subunit is a glutamic acid or glutamine. In exemplified embodiments, the mutant plant AGPase small subunit comprises the
15 amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7, or a fragment or variant thereof. In specific embodiments, the polynucleotide comprises the nucleotide sequences shown in SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13, or a fragment or variant thereof.

In another embodiment, a polynucleotide encodes a chimeric plant AGPase small
20 subunit composed of sequences from two different plants (as described in U.S. Patent No. 7,173,165) and also comprising an amino acid mutation of the invention wherein the threonine amino acid corresponding to amino acid position 462 of wild type maize endosperm AGPase small subunit is substituted with an amino acid that confers increased heat stability. In a specific embodiment, the amino acid substituted is an isoleucine. In
25 one embodiment, the chimeric AGPase small subunit comprises a C-terminal portion from one plant and an N-terminal portion from another plant. In one embodiment, a chimeric protein of the present invention comprises an N-terminus sequence having approximately the first 150 to 250 amino acids of the N-terminus of a first plant AGPase small subunit and a C-terminus sequence comprising approximately the terminal 300
30 residues or less of the C-terminus of a second plant AGPase small subunit. Thus, the C-terminus of the chimeric subunit can comprise the terminal 300, or 299, or 298, or 297, or 296, or 295, and so forth, residues of the C-terminus of the second plant. The subunit

sequences can be from an AGPase of a monocot or dicot plant, or both a monocot and a dicot. Monocotyledonous plants, such as, for example, rice, wheat, barley, oats, sorghum, maize, lilies, and millet are included within the scope of the invention. Dicot plants can include, for example, tobacco, soybean, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce. In one embodiment, the first 200 or so amino acids of the N-terminus of the chimeric protein are from the N-terminus of maize endosperm AGPase small subunit and the C-terminus amino acids are from the C-terminus of potato tuber AGPase small subunit and include the mutation corresponding to amino acid position 462 of the present invention. In a specific embodiment, the C-terminus region of a chimeric protein of the present invention comprises the terminal 276 amino acids of the AGPase small subunit of potato tuber. In an exemplified embodiment, the chimeric protein comprises a portion of the small subunit of maize endosperm AGPase and a portion of the small subunit of potato tuber AGPase. In a specific embodiment, the chimeric protein contains a) the first 199 amino acids (*i.e.*, amino acids 1 through 199) from the small subunit of maize endosperm AGPase and the carboxyl terminal end of the small subunit of potato tuber AGPase, starting at amino acid 246 (*i.e.*, amino acids 246 through 521) using the amino acid sequence shown for the protein deposited as Genbank accession number X61186 (or, alternatively, starting at amino acid 175 using the numbering system for the potato AGPase subunit as in Hannah *et al.*, 2001) and b) the mutation corresponding to amino acid position 462 of the present invention. In an exemplified embodiment, the plant chimeric AGPase small subunit comprises the amino acid sequence shown in SEQ ID NO:4, or a fragment or variant thereof. In a specific embodiment, the polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:3, or a fragment or variant thereof. In another embodiment, the polynucleotide encodes a mutant plant AGPase small subunit that can additionally comprise an amino acid mutation described in published International patent application WO 2005/019425 (Hannah and Linebarger). In a further embodiment, the mutant AGPase small subunit encoded by the polynucleotide also comprises an amino acid mutation wherein a tyrosine at position 36 is substituted with a cysteine. The mutant AGPase small subunit can also optionally comprise an amino acid inserted between the serine and threonine amino acids at positions 34 and 35, respectively. In specific embodiments, the amino acid inserted between position 34 and 35 of the mutant AGPase small subunit is a glutamic acid or

glutamine. In exemplified embodiments, the mutant plant AGPase small subunit comprises the amino acid sequence shown in SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10, or a fragment or variant thereof. In specific embodiments, the polynucleotide comprises the nucleotide sequences shown in SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or a fragment or variant thereof.

The subject invention also concerns methods for increasing heat stability and/or increasing starch biosynthesis, and increasing crop yield of a plant or plant tissue. In one embodiment, a method of the invention comprises introducing one or more polynucleotides of the present invention into a plant. In certain embodiments, the polynucleotides introduced into the plant encode one or more polypeptides comprising the amino acid sequence shown in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a fragment or variant thereof. In a specific embodiment, the polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or a fragment or variant thereof. In further specific embodiments, the polynucleotide comprises the nucleotide sequences shown in SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or a fragment or variant thereof. In one embodiment, the polynucleotide is stably incorporated into the genome of the plant or plant tissue. The polynucleotide can comprise regulatory elements, such as a promoter and/or enhancer sequences, that provide for increased expression of the polynucleotide and/or the polypeptide encoded thereby. In a specific embodiment, the promoter sequence is one that provides for constitutive or tissue-specific (*e.g.*, endosperm) expression. Plants or plant tissues containing the polynucleotide, or progeny of the plants, optionally can be screened for increased expression of a polynucleotide or polypeptide of the invention. In one embodiment, multiple copies of one or more polynucleotides of the invention are introduced into a plant or plant tissue and stably incorporated into the genome of the plant. In one embodiment, a polynucleotide of the invention is provided in an expression construct as described herein.

The subject invention also concerns mutant AGPase small subunit polypeptides encoded by the polynucleotides of the invention. In one embodiment, the polypeptide comprises the amino acid sequence shown in SEQ ID NO:2, or a fragment or variant

thereof. In other embodiments, the polypeptide comprises the amino acid sequence shown in SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a fragment or variant thereof. In another embodiment, the polypeptide comprises the amino acid sequence shown in SEQ ID NO:4, or a fragment or variant thereof. In still a further embodiment, the polypeptide comprises the amino acid sequence shown in any of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10, or a fragment or variant thereof.

The subject invention also concerns mutant plant AGPase enzymes comprising one or more mutant AGPase small subunit polypeptides of the invention. The mutant plant AGPase can also comprise one or more wild type AGPase large subunit polypeptides. In specific embodiments, a mutant plant AGPase enzyme comprises one or more mutant AGPase small subunit polypeptides any of which can comprise the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a fragment or variant of any such sequence, wherein the mutant AGPase enzyme exhibits increased heat stability relative to a wild type AGPase enzyme. In one embodiment, the mutant plant enzyme comprises two mutant AGPase small subunits of the invention, wherein the mutant polypeptides can have the same mutation(s) or can have different mutation(s). The subject invention also concerns mutant plant AGPase enzymes comprising one or more mutant AGPase small subunit polypeptides of the invention and one or more mutant AGPase large subunit polypeptides. In one embodiment, the mutant AGPase large subunit polypeptide can be any of those as described in any of U.S. Patent Nos. 5,589,618; 5,650,557; 5,872,216; 6,069,300; 6,184,438; 6,403,863; 6,809,235; 7,173,165; 7,312,378; and 6,969,783. In one embodiment, a mutant AGPase large subunit polypeptide comprises a *Rev6* mutation. In another embodiment, a mutant AGPase large subunit polypeptide comprises one or more heat stable (HS) mutations, as described in U.S. Patent Nos. 6,069,300; 6,403,863; 6,809,235; 7,312,378; and 6,969,783, and published International patent application nos. WO 99/58698; WO 2003/0070901; WO 98/22601; and WO 02/072784, such as, for example, the HS33 mutation. In one embodiment, the mutant plant AGPase enzyme comprises two mutant AGPase small subunit polypeptides of the invention, wherein the mutant AGPase small subunit polypeptides can have the same mutation(s) or can have

different mutation(s), as described herein. In another embodiment, the mutant plant AGPase enzyme comprises two mutant AGPase large subunit polypeptides wherein the mutant AGPase large subunit polypeptides can have the same mutation(s) or can have different mutation(s). In a further embodiment, the mutant plant AGPase enzyme
5 comprises two mutant AGPase small subunit polypeptides of the invention and two mutant SH2 polypeptides, wherein the mutant AGPase small subunit polypeptides and the mutant AGPase large subunit polypeptides can have the same mutation(s) or can have different mutation(s), as described herein.

The subject invention also concerns methods for providing for a mutant plant
10 AGPase enzyme having increased heat stability relative to wild type plant AGPase. In one embodiment, the method comprises incorporating or providing one or more mutant AGPase small subunit polypeptides of the present invention with wild type or mutant AGPase large subunits in an AGPase enzyme. In one embodiment, the AGPase enzyme comprises a tetramer of polypeptide subunits, wherein one, two, or more of the subunits is
15 a mutant polypeptide of the present invention. In one embodiment, the AGPase enzyme also comprises a mutant AGPase large subunit polypeptide subunit, such as a mutant large subunit comprising a *Rev6* and/or a heat stability mutation, such as HS33.

The subject invention also concerns plants, plant tissue, and plant cells of the invention that comprise a polynucleotide or the protein encoded by the polynucleotide of
20 the invention, or that express a mutant polypeptide of the invention, or a fragment or variant thereof, or that comprise or express a mutant plant AGP enzyme of the present invention. Plant tissue includes, but is not limited to, seed, scion, and rootstock. Plants within the scope of the present invention include monocotyledonous plants, such as, for example, rice, wheat, barley, oats, rye, sorghum, maize, sugarcane, pineapple, onion,
25 bananas, coconut, lilies, turfgrasses, and millet. Plants within the scope of the present invention also include dicotyledonous plants, such as, for example, tomato, cucumber, squash, peas, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, beans, tobacco, potato, sweet potato, yams, cassava, radish, broccoli, spinach, cabbage, rape, apple trees, citrus (including oranges, mandarins, grapefruit, lemons, limes and the like),
30 grape, cotton, sunflower, strawberry, lettuce, and hop. Herb plants containing a polynucleotide of the invention are also contemplated within the scope of the invention. Herb plants include parsley, sage, rosemary, thyme, and the like. In one embodiment, the

plant, plant tissue, or plant cell is *Zea mays*. In one embodiment, a plant, plant tissue, or plant cell is a transgenic plant, plant tissue, or plant cell. In another embodiment, a plant, plant tissue, or plant cell is one that has been obtained through a breeding program.

Polynucleotides useful in the present invention can be provided in an expression
5 construct. Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription
10 termination sequences, translation termination sequences, enhancers, and polyadenylation elements. As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to
15 function in their intended manner. In general, operably linked components are in contiguous relation.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a mutant polypeptide of the invention. Promoters can be incorporated into a polynucleotide using standard techniques
20 known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site in the expression construct as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A
25 transcription start site is typically included in the expression construct.

If the expression construct is to be provided in or introduced into a plant cell, then plant viral promoters, such as, for example, a cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or a CaMV 19S promoter or a cassava vein mosaic can be used. Other
30 promoters that can be used for expression constructs in plants include, for example, prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1'- or 2'-promoter of *A. tumefaciens*, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from

petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu *et al.*, 1993), maize WipI promoter, maize trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Tissue-specific promoters, for example fruit-specific promoters, such as the E8 promoter of tomato (accession number: AF515784; Good *et al.* (1994)) can be used. Fruit-specific promoters such as flower organ-specific promoters can be used with an expression construct of the present invention for expressing a polynucleotide of the invention in the flower organ of a plant. Examples of flower organ-specific promoters include any of the promoter sequences described in U.S. Patent Nos. 6,462,185; 5,639,948; and 5,589,610. Seed-specific promoters such as the promoter from a β -phaseolin gene (for example, of kidney bean) or a glycinin gene (for example, of soybean), and others, can also be used. Endosperm-specific promoters include, but are not limited to, MEG1 (EPO application No. EP1528104) and those described by Wu *et al.* (1998), Furtado *et al.* (2001), and Hwang *et al.* (2002). Root-specific promoters, such as any of the promoter sequences described in U.S. Patent No. 6,455,760 or U.S. Patent No. 6,696,623, or in published U.S. patent application Nos. 20040078841; 20040067506; 20040019934; 20030177536; 20030084486; or 20040123349, can be used with an expression construct of the invention. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), developmentally-regulated promoters, and inducible promoters (such as those promoters that can be induced by heat, light, hormones, or chemicals) are also contemplated for use with polynucleotide expression constructs of the invention.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, a sequence encoding a signal peptide, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. A signal peptide sequence is a short amino acid sequence typically present at the amino terminus of a protein that is responsible for the relocation of an operably linked mature polypeptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting gene products to an intended cellular

and/or extracellular destination through the use of an operably linked signal peptide sequence is contemplated for use with the polypeptides of the invention. Classical enhancers are cis-acting elements that increase gene transcription and can also be included in the expression construct. Classical enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element. Intron-mediated enhancer elements that enhance gene expression are also known in the art. These elements must be present within the transcribed region and are orientation dependent. Examples include the maize *shrunk-1* enhancer element (Clancy and Hannah, 2002).

DNA sequences which direct polyadenylation of mRNA transcribed from the expression construct can also be included in the expression construct, and include, but are not limited to, an octopine synthase or nopaline synthase signal. The expression constructs of the invention can also include a polynucleotide sequence that directs transposition of other genes, *i.e.*, a transposon.

Polynucleotides of the present invention can be composed of either RNA or DNA. Preferably, the polynucleotides are composed of DNA. The subject invention also encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein. Polynucleotides and polypeptides of the invention can be provided in purified or isolated form.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode mutant polypeptides of the present invention. A table showing all possible triplet codons (and where U also stands for T) and the amino acid encoded by each codon is described in Lewin (1985). 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, mutant polypeptides 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 the polynucleotides of the present invention. Allelic variants of the nucleotide sequences

encoding a wild type or mutant polypeptide of the invention are also encompassed within the scope of the invention.

Substitution of amino acids other than those specifically exemplified or naturally present in a wild type or mutant polypeptide and/or AGPase enzyme of the invention are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino acids of a mutant AGPase small subunit polypeptide, so long as the mutant polypeptide having the substituted amino acids retains substantially the same functional activity as the mutant polypeptide in which amino acids have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ -amino butyric acid, ϵ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form. Allelic variants of a protein sequence of a wild type or mutant AGPase small or large subunit polypeptide of the present invention are also encompassed within the scope of the invention.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a mutant AGPase small subunit polypeptide of the present invention and/or a wild type or mutant AGPase large subunit polypeptide having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the polypeptide having the substitution still retains substantially the same functional activity (*e.g.*, enzymatic and/or increased heat stability of an AGPase enzyme) as the polypeptide that does not have the substitution. Polynucleotides encoding a mutant AGPase small subunit polypeptide and/or a wild type or mutant AGPase large subunit polypeptide having one or more amino acid substitutions in the sequence are contemplated within the

scope of the present invention. Table 1 below provides a listing of examples of amino acids belonging to each class.

Table 1.	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

The subject invention also concerns variants of the polynucleotides of the present invention that encode functional wild type or mutant AGPase small or large subunit polypeptides of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Fragments and variants of a mutant polypeptide of the present invention can be generated as described herein and tested for the presence of enzymatic and heat stable function using standard techniques known in the art. Thus, an ordinarily skilled artisan can readily prepare and test fragments and variants of a mutant polypeptide of the invention and determine whether the fragment or variant retains functional activity relative to full-length or a non-variant mutant polypeptide.

Polynucleotides and polypeptides contemplated within the scope of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those sequences of the invention specifically exemplified herein. The sequence

identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCBI/NIH website.

The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.*, 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (T_m) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, T_m , is described by the following formula (Beltz *et al.*, 1983):

$$T_m = 81.5 \text{ } ^\circ\text{C} + 16.6 \text{ Log}[\text{Na}^+] + 0.41(\%G+C) - 0.61(\% \text{ formamide}) - 600/\text{length of duplex in base pairs.}$$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).
- (2) Once at $T_m - 20 \text{ } ^\circ\text{C}$ for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

As used herein, the terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide, ribonucleotide, or a mixed deoxyribonucleotide and ribonucleotide

polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include the DNA strand sequence that is transcribed into RNA and the strand sequence that is complementary to the DNA strand that is transcribed. The polynucleotide sequences also include both full-length sequences as well as shorter sequences derived from the full-length sequences. Allelic variations of the exemplified sequences also fall within the scope of the subject invention. The polynucleotide sequence includes both the sense and antisense strands either as individual strands or in the duplex.

Techniques for transforming plant cells with a gene are known in the art and include, for example, *Agrobacterium* infection, biolistic methods, electroporation, calcium chloride treatment, PEG-mediated transformation, *etc.* U.S. Patent No. 5,661,017 teaches methods and materials for transforming an algal cell with a heterologous polynucleotide. Transformed cells can be selected, redifferentiated, and grown into plants that contain and express a polynucleotide of the invention using standard methods known in the art. The seeds and other plant tissue and progeny of any transformed or transgenic plant cells or plants of the invention are also included within the scope of the present invention.

The subject invention also concerns methods for producing a plant that exhibits increased heat stability relative to a wild type plant, wherein a polynucleotide encoding a mutant AGPase small subunit polypeptide of the present invention is introduced into a plant cell and the polypeptide(s) encoded by the polynucleotide(s) is expressed. In one embodiment, the plant cell comprises non-mutant genes encoding wild type AGPase large subunit polypeptide. In another embodiment, the plant cell comprises at least one polynucleotide encoding a mutant AGPase large subunit polypeptide. In a further embodiment, a polynucleotide encoding a mutant AGPase large subunit polypeptide is also introduced into a plant cell along with the polynucleotide encoding the mutant AGPase small subunit polypeptide. In one embodiment, the polynucleotide or polynucleotides is incorporated into the genome of the plant cell and a plant is grown from the plant cell. In a preferred embodiment, the plant grown from the plant cell stably expresses the incorporated polynucleotide or polynucleotides.

The subject invention also concerns oligonucleotide probes and primers, such as polymerase chain reaction (PCR) primers, that can hybridize to a coding or non-coding sequence of a polynucleotide of the present invention. Oligonucleotide probes of the invention can be used in methods for detecting and quantitating nucleic acid sequences encoding a mutant AGPase small subunit polypeptide of the invention. Oligonucleotide primers of the invention can be used in PCR methods and other methods involving nucleic acid amplification. In a preferred embodiment, a probe or primer of the invention can hybridize to a polynucleotide of the invention under stringent conditions. Probes and primers of the invention can optionally comprise a detectable label or reporter molecule, such as fluorescent molecules, enzymes, radioactive moiety (*e.g.*, ^3H , ^{35}S , ^{125}I , *etc.*), and the like. Probes and primers of the invention can be of any suitable length for the method or assay in which they are being employed. Typically, probes and primers of the invention will be 10 to 500 or more nucleotides in length. Probes and primers that are 10 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, 91 to 100 or more nucleotides in length are contemplated within the scope of the invention. Probes and primers of the invention can have complete (100%) nucleotide sequence identity with the polynucleotide sequence, or the sequence identity can be less than 100%. For example, sequence identity between a probe or primer and a sequence can be 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70% or any other percentage sequence identity so long as the probe or primer can hybridize under stringent conditions to a nucleotide sequence of a polynucleotide of the invention. In one embodiment, a probe or primer of the invention has 70% or greater, 75% or greater, 80% or greater, 85% or greater, 90% or greater, or 95% to 100% sequence identity with a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or the complement thereof.

The subject invention also concerns isolated mutant AGPase small subunit polypeptides. In one embodiment, the mutant AGPase small subunit polypeptide is an AGPase small subunit polypeptide of *Zea mays*. In a specific embodiment, an AGPase small subunit polypeptide of the invention has an amino acid sequence as shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or functional fragment or variant thereof. An AGPase small

subunit polypeptide or enzyme of the invention can be purified using standard techniques known in the art. In one embodiment, a polynucleotide of the invention encoding an AGPase small subunit polypeptide is incorporated into a microorganism, such as *E. coli*, and the AGPase small subunit polypeptide expressed in the microorganism and then
5 isolated therefrom.

In certain embodiments, polypeptides of the invention, and functional peptide fragments thereof, can be used to generate antibodies that bind specifically to a polypeptide of the invention, and such antibodies are contemplated within the scope of the invention. The antibodies of the invention can be polyclonal or monoclonal and can
10 be produced and isolated using standard methods known in the art.

Polypeptide fragments according to the subject invention typically comprise a contiguous span of about or at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86,
15 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179,
20 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269,
25 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359,
30 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413,

414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, or 475 amino acids of SEQ ID NO:2, SEQ ID NO:4, 5 SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10.

In certain embodiments, polypeptide fragments of the subject invention can be any integer in length from at least about 25 consecutive amino acids to 1 amino acid less than the full-length sequence, such as those shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID 10 NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10. Thus, for SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10, a polypeptide fragment can be any integer of consecutive amino acids from about 25 to 475 amino acids. The term "integer" is used herein in its mathematical sense and thus representative integers include: 25, 26, 15 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 20 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 25 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 30 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368,

369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, and/or 475.

Each polypeptide fragment of the subject invention can also be described in terms of its N-terminal and C-terminal positions. For example, combinations of N-terminal to C-terminal fragments of about 25 contiguous amino acids to 1 amino acid less than the full length polypeptide, such as those of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 are included in the present invention. Thus, using SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 as an example, a 25 consecutive amino acid fragment could correspond to amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 selected from the group consisting of 1-25, 2-26, 3-27, 4-28, 5-29, 6-30, 7-31, 8-32, 9-33, 10-34, 11-35, 12-36, 13-37, 14-38, 15-39, 16-40, 17-41, 18-42, 19-43, 20-44, 21-45, 22-46, 23-47, 24-48, 25-49, 26-50, 27-51, 28-52, 29-53, 30-54, 31-55, 32-56, 33-57, 34-58, 35-59, 36-60, 37-61, 38-62, 39-63, 40-64, 41-65, 42-66, 43-67, 44-68, 45-69, 46-70, 47-71, 48-72, 49-73, 50-74, 51-75, 52-76, 53-77, 54-78, 55-79, 56-80, 57-81, 58-82, 59-83, 60-84, 61-85, 62-86, 63-87, 64-88, 65-89, 66-90, 67-91, 68-92, 69-93, 70-94, 71-95, 72-96, 73-97, 74-98, 75-99, 76-100, 77-101, 78-102, 79-103, 80-104, 81-105, 82-106, 83-107, 84-108, 85-109, 86-110, 87-111, 88-112, 89-113, 90-114, 91-115, 92-116, 93-117, 94-118, 95-119, 96-120, 97-121, 98-122, 99-123, 100-124, 101-125, 102-126, 103-127, 104-128, 105-129, 106-130, 107-131, 108-132, 109-133, 110-134, 111-135, 112-136, 113-137, 114-138, 115-139, 116-140, 117-141, 118-142, 119-143, 120-144, 121-145, 122-146, 123-147, 124-148, 125-149, 126-150, 127-151, 128-152, 129-153, 130-154, 131-155, 132-156, 133-157, 134-158, 135-159, 136-160, 137-161, 138-162, 139-163, 140-164, 141-165, 142-166, 143-167, 144-168, 145-169, 146-170, 147-171, 148-172, 149-173, 150-174, 151-175, 152-176, 153-177, 154-178, 155-179, 156-180, 157-181, 158-182, 159-183, 160-184, 161-185, 162-186,

163-187, 164-188, 165-189, 166-190, 167-191, 168-192, 169-193, 170-194, 171-195,
172-196, 173-197, 174-198, 175-199, 176-200, 177-201, 178-202, 179-203, 180-204,
181-205, 182-206, 183-207, 184-208, 185-209, 186-210, 187-211, 188-212, 189-213,
190-214, 191-215, 192-216, 193-217, 194-218, 195-219, 196-220, 197-221, 198-222,
5 199-223, 200-224, 201-225, 202-226, 203-227, 204-228, 205-229, 206-230, 207-231,
208-232, 209-233, 210-234, 211-235, 212-236, 213-237, 214-238, 215-239, 216-240,
217-241, 218-242, 219-243, 220-244, 221-245, 222-246, 223-247, 224-248, 225-249,
226-250, 227-251, 228-252, 229-253, 230-254, 231-255, 232-256, 233-257, 234-258,
235-259, 236-260, 237-261, 238-262, 239-263, 240-264, 241-265, 242-266, 243-267,
10 244-268, 245-269, 246-270, 247-271, 248-272, 249-273, 250-274, 251-275, 252-276,
253-277, 254-278, 255-279, 256-280, 257-281, 258-282, 259-283, 260-284, 261-285,
262-286, 263-287, 264-288, 265-289, 266-290, 267-291, 268-292, 269-293, 270-294,
271-295, 272-296, 273-297, 274-298, 275-299, 276-300, 277-301, 278-302, 279-303,
280-304, 281-305, 282-306, 283-307, 284-308, 285-309, 286-310, 287-311, 288-312,
15 289-313, 290-314, 291-315, 292-316, 293-317, 294-318, 295-319, 296-320, 297-321,
298-322, 299-323, 300-324, 301-325, 302-326, 303-327, 304-328, 305-329, 306-330,
307-331, 308-332, 309-333, 310-334, 311-335, 312-336, 313-337, 314-338, 315-339,
316-340, 317-341, 318-342, 319-343, 320-344, 321-345, 322-346, 323-347, 324-348,
325-349, 326-350, 327-351, 328-352, 329-353, 330-354, 331-355, 332-356, 333-357,
20 334-358, 335-359, 336-360, 337-361, 338-362, 339-363, 340-364, 341-365, 342-366,
343-367, 344-368, 345-369, 346-370, 347-371, 348-372, 349-373, 350-374, 351-375,
352-376, 353-377, 354-378, 355-379, 356-380, 357-381, 358-382, 359-383, 360-384,
361-385, 362-386, 363-387, 364-388, 365-389, 366-390, 367-391, 368-392, 369-393,
370-394, 371-395, 372-396, 373-397, 374-398, 375-399, 376-400, 377-401, 378-402,
25 379-403, 380-404, 381-405, 382-406, 383-407, 384-408, 385-409, 386-410, 387-411,
388-412, 389-413, 390-414, 391-415, 392-416, 393-417, 394-418, 395-419, 396-420,
397-421, 398-422, 399-423, 400-424, 401-425, 402-426, 403-427, 404-428, 405-429,
406-430, 407-431, 408-432, 409-433, 410-434, 411-435, 412-436, 413-437, 414-438,
415-439, 416-440, 417-441, 418-442, 419-443, 420-444, 421-445, 422-446, 423-447,
30 424-448, 425-449, 426-450, 427-451, 428-452, 429-453, 430-454, 431-455, 432-456,
433-457, 434-458, 435-459, 436-460, 437-461, 438-462, 439-463, 440-464, 441-465,
442-466, 443-467, 444-468, 445-469, 446-470, 447-471, 448-472, 449-473, 450-474,

and/or 451-475. Similarly, the amino acids corresponding to all other fragments of sizes between 26 consecutive amino acids and 474 (or 475) consecutive amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 are included in the present invention and can also be immediately envisaged based on these examples. Therefore, additional examples, illustrating various fragments of the polypeptides of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 are not individually listed herein in order to avoid unnecessarily lengthening the specification.

Polypeptide fragments comprising: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447,

448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, and 474 (or 475) consecutive amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10 may alternatively be described by the formula “n to c” (inclusive), where “n” equals the N-terminal amino acid position and “c” equals the C-terminal amino acid position of the polypeptide. In this embodiment of the invention, “n” is an integer having a lower limit of 1 and an upper limit of the total number of amino acids of the full length polypeptide minus 24 (e.g., 475-24=451 for SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:8; 476-24=452 for SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:10). “c” is an integer between 25 and the number of amino acids of the full length polypeptide sequence (475 for SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:8; 476 for SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:10) and “n” is an integer smaller than “c” by at least 24. Therefore, for SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10, “n” is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334,

335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 5 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, and 451 (or 452); and “c” is any integer selected from the group consisting of: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 10 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 15 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 20 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 25 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 30 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, and 475 (or 476) provided that “n” is a value less than “c” by at

least 24. Every combination of “n” and “c” positions are included as specific embodiments of polypeptide fragments of the invention. All ranges used to describe any polypeptide fragment embodiment of the present invention are inclusive unless specifically set forth otherwise.

5 Fragments of a mutant AGPase small subunit polypeptide of the invention or an AGPase large subunit polypeptide, as described herein, can be obtained by cleaving the polypeptides of the invention with a proteolytic enzyme (such as trypsin, chymotrypsin, or collagenase) or with a chemical reagent, such as cyanogen bromide (CNBr). Alternatively, polypeptide fragments can be generated in a highly acidic environment, for
10 example at pH 2.5. Polypeptide fragments can also be prepared by chemical synthesis or using host cells transformed with an expression vector comprising a polynucleotide encoding a fragment of an AGPase large subunit polypeptide or a fragment of a mutant AGPase small subunit polypeptide of the invention, for example, a mutant polypeptide that is a fragment of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4,
15 SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25. Fragments of a mutant large or small subunit AGPase polypeptide of the invention also contemplated herein include fragments of the polypeptide wherein all or a part of a transit or signal sequence of the polypeptide is removed.

20 The subject invention also concerns cells transformed with a polynucleotide of the present invention encoding a mutant AGPase small subunit polypeptide of the invention. In one embodiment, the cell is transformed with a polynucleotide sequence comprising a sequence encoding the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10,
25 SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a functional fragment or variant thereof. In a specific embodiment, the cell is transformed with a polynucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or a sequence encoding a functional fragment or variant of SEQ ID NO:2, SEQ ID NO:4, SEQ
30 ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25. In one embodiment, a cell is also transformed with a polynucleotide encoding a mutant

AGPase large subunit polypeptide as described herein. In one embodiment, the polynucleotide sequence is provided in an expression construct of the invention. The transformed cell can be a prokaryotic cell, for example, a bacterial cell such as *E. coli* or *B. subtilis*, or the transformed cell can be a eukaryotic cell, for example, a plant cell, including protoplasts, or an animal cell. Plant cells include, but are not limited to, dicotyledonous, monocotyledonous, and conifer cells. In one embodiment, the plant cell is a cell from a *Zea mays* plant. Animal cells include human cells, mammalian cells, avian cells, and insect cells. Mammalian cells include, but are not limited to, COS, 3T3, and CHO cells.

10 The subject invention also concerns methods for increasing starch synthesis in a plant or plant tissue (such as a plant seed or endosperm tissue). In one embodiment, a method of the invention comprises introducing one or more polynucleotides of the present invention into a plant. In certain embodiments, the polynucleotides introduced into the plant encode one or more polypeptides comprising the amino acid sequence shown in any
15 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a fragment or variant thereof. In a specific embodiment, the polynucleotide comprises the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or a fragment or variant thereof. In further specific
20 embodiments, the polynucleotide comprises the nucleotide sequences shown in SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or a fragment or variant thereof. In one embodiment, the polynucleotide is stably incorporated into the genome of the plant or plant tissue. The polynucleotide can comprise regulatory elements, such as a promoter and/or enhancer sequences, that
25 provide for increased expression of the polynucleotide and/or the polypeptide encoded thereby. In a specific embodiment, the promoter sequence is one that provides for constitutive or tissue-specific (*e.g.*, endosperm) expression. Plants or plant tissues containing the polynucleotide, or progeny of the plants, optionally can be screened for increased expression of a polynucleotide or polypeptide of the invention. In one
30 embodiment, multiple copies of one or more polynucleotides of the invention are introduced into a plant or plant tissue and stably incorporated into the genome of the

plant. In one embodiment, a polynucleotide of the invention is provided in an expression construct as described herein.

Single letter amino acid abbreviations are defined in Table 2.

Table 2.			
Letter Symbol	Amino Acid	Letter Symbol	Amino Acid
A	Alanine	M	Methionine
B	Asparagine or aspartic acid	N	Asparagine
C	Cysteine	P	Proline
D	Aspartic Acid	Q	Glutamine
E	Glutamic Acid	R	Arginine
F	Phenylalanine	S	Serine
G	Glycine	T	Threonine
H	Histidine	V	Valine
I	Isoleucine	W	Tryptophan
K	Lysine	Y	Tyrosine
L	Leucine	Z	Glutamine or glutamic acid

5

MATERIALS AND METHODS

Random mutagenesis

Mutations were introduced into *Sh2* and *Bt2* by PCR random mutagenesis (GeneMorph II EZClone Domain Mutagenesis Kit, Stratagene). A mixture of non-biased, error-prone DNA polymerases was used to introduce point mutations. Wildtype *Sh2* and *Bt2* coding sequences in pMONc*Sh2* and pMONc*Bt2* (Giroux *et al.*, 1996) respectively were used as templates for PCR. Two pairs of primers (*Sh2*: 5'-GAAGGAGATATATCCATGG-3' (SEQ ID NO:17), 5'-GGATCCCCGGGTACCGAGCTC-3' (SEQ ID NO:18) *Bt2*: 5'-GAAGGAGATATATCCATGG-3' (SEQ ID NO:19), 5'-GTTGATATCTGAATTCGAGCTC-3' (SEQ ID NO:20)) flanking *Sh2* and *Bt2* were used for error-prone PCR. Mutant *Sh2* clones produced by PCR were subcloned into vector pMONc*Sh2* according to Stratagene protocols. pMONc*Sh2* was then used to transform *E. coli* strain AC70R1-504 that contained wildtype *Bt2* in the compatible vector pMONc*Bt2*. Mutant *Bt2* clones produced by PCR were subcloned into vector pMONc*Bt2*.

20

pMONcBt2 was then used to transform *E.coli* strain AC70R1-504 that contained wildtype *Sh2* in the compatible vector pMONcSh2.

Bacterial Expression System

A bacterial expression system (Iglesias *et al.*, 1993) allowed us to randomly mutagenize maize endosperm AGPase genes and score AGPase activity in a fast and efficient way by exposing plates to iodine vapors as described below. The *E. coli* system is ideal for studying plant AGPases for a number of reasons discussed in Georgelis *et al.* (2007).

Glycogen Detection

Glycogen synthesis was detected by production of brown staining colonies following exposure to iodine vapors. *E.coli* cells were grown on Kornberg media in the presence of 75 µg/mL spectinomycin, 50 µg/mL kanamycin and 1% w/v glucose for 16 h at 37°C (Govons *et al.*, 1969). The colonies were exposed to iodine vapors for 1 min. Colonies with inactive AGPase produced no color following exposure to iodine vapors while active AGPase produced glycogen and, in turn, brown staining with iodine. AGPase variants staining darker than wildtype were selected for further study.

Glycogen Quantitation

Glycogen quantitation was performed by phenol reaction (Hanson and Phillips, 1981). In brief, glycogen was extracted from 1.6 ml of *E.coli* cells (OD₆₀₀=2.0) grown in LB containing 2% w/v glucose by boiling for 3 hours in 50% w/v KOH. Glycogen was then precipitated by adding ethanol to 70% v/v and centrifuging at 10000 x g for 10 min. After pellet drying, 200 µl de-ionized water, 200 µl of 5% w/v phenol and 1 mL of concentrated sulfuric acid were added. Glycogen was estimated by the absorbance at 488 nm.

DNA Sequencing

Sh2 and *Bt2* mutants that produced enhanced glycogen were double-pass sequenced by the Genome Sequencing Services Laboratory (GSSL) of the Interdisciplinary Center for Biotechnology Research at the University of Florida. Data analysis was performed by Bioedit software (Hall, 1999).

Purification of Maize Endosperm AGPase from AC70R1-504 *E. coli* Cells

AC70R1-504 *E.coli* cells expressing maize endosperm AGPase were grown in 2 L of Luria-Broth (LB) medium in the presence of 75 µg/mL spectinomycin, 50 µg/mL

kanamycin and 2% w/v glucose for 16h at 37°C with shaking. At OD₆₀₀=0.6, 0.2mM isopropyl-beta-D-thiogalactoside (IPTG) and 0.02mg/mL nalidixic acid were added to induce protein expression. The cultures were immediately moved to room temperature and grown for 4h with shaking. The following steps were conducted at 4°C. Cells were
5 harvested by centrifuging at 3000 x g and the pellet was resuspended in 16mL of buffer A (50mM KH₂PO₄ pH 7.0, 5mM MgCl₂, 0.5mM EDTA) and protease inhibitors (1µg/mL pepstatin, 0.1mM PMSF, 10 µg/mL chymostatin, and 1mM benzamidine). The cells were lysed with a French press and centrifuged at 26000 x g. The protein concentration of the supernatant was adjusted to 30mg/mL by adding buffer A. Three tenths of volume of 1%
10 protamine sulfate were added and the mixture stirred on ice for 20 min and then centrifuged at 26000 x g for 20min. The supernatant was brought to 45% saturation with ammonium sulfate, stirred on ice for 20min and centrifuged at 26000 x g for 20min. The pellet was re-suspended in 2-2.5mL of buffer A. The mixture was passed through a strong anion exchange column (macro-prep High Q support, Biorad), and an Econo-pac hydroxyapatite cartridge (Biorad) as described by Boehlein *et al.* (2005). AGPase was
15 desalted by using Zeba Micro Desalt Spin Columns (Pierce) before assaying. AGPase was exchanged into 50mM HEPES, 5mM MgCl₂, 0.5mM EDTA and 0.5mg/mL BSA (for stability).

Kinetic Characterization of AGPase

20 The forward direction of the reaction was used ($G-1-P + ATP \rightarrow ADP-glucose + PPi$) for estimating k_{cat} , K_m for ATP and G-1-P, and affinities for 3-PGA and Pi. More specifically, 0.04-0.06µg of purified AGPase was assayed for specific activity in a total volume of 200µl of 50mM HEPES pH 7.4, 15mM MgCl₂, 1.0mM ATP, and 2.0mM G-1-P at 37°C for 10min. For determining K_m s for ATP and G-1-P and K_a for 3-PGA, varying
25 amounts of ATP, G-1-P and 3-PGA respectively. K_i for Pi was estimated by adding various amounts of Pi, 1mM ATP, 2mM G-1-P, and 2.5mM 3-PGA were used. The reaction was stopped by boiling for 2min. PPi was estimated by coupling the reaction to a reduction in NADH concentration using 300µl of coupling reagent. The coupling reagent contained 25mM imidazole pH 7.4, 4mM MgCl₂, 1mM EDTA, 0.2mM NADH, 0.725U
30 aldolase, 0.4U triose phosphate isomerase, 0.6U glycerophosphate dehydrogenase, 1mM fructose 6-phosphate and 0.8µg of pyrophosphate dependent phosphofructokinase (PPi-PFK). All the enzymes were purchased from Sigma except for PPi-PFK which was

produced and purified according to Deng *et al.* 1999 with some modifications (Boehlein and Hannah, unpublished data). NADH concentration was estimated by absorbance at 340nm. PPi concentration was calculated by a standard curve developed by using various amounts of PPi instead of AGPase. The amount of PPi produced by AGPase was linear
5 with time and enzyme concentration. The kinetic constants of AGPase were calculated by Prism 4.0 (Graph Pad, San Diego CA).

Measuring AGPase Specific Activity from Crude or Partially Purified Protein Extracts

AC70R1-504 *E.coli* cells expressing maize endosperm AGPase were grown in 2 L
10 of Luria-Broth (LB) medium in the presence of 75 µg/mL spectinomycin, 50 µg/mL kanamycin and 2% w/v glucose at 37°C with shaking until OD₆₀₀=2.0. Gene expression was not induced. The following steps were conducted at 4°C. Cells were harvested by centrifuging at 3000 x g and the pellet was resuspended in 16mL of buffer A (50mM KH₂PO₄ pH 7.0, 5mM MgCl₂, 0.5mM EDTA) and protease inhibitors (1µg/mL pepstatin,
15 0.1mM PMSF, 10 µg/mL chymostatin, and 1mM benzamidine). The cells were lysed with a French press and centrifuged at 26000 x g. AGPase activity of the crude extract was measured from the supernatant stored at -80°C. The rest of the supernatant was partially purified through protamine sulfate and ammonium sulfate as described above. Protein extracts were desalted as described by Boehlein *et al.* (2005) before assay and
20 were exchanged into 50mM HEPES, 5mM MgCl₂, and 0.5mM EDTA. AGPase specific activity was monitored in the reverse direction (ADP-glucose + PPi → G-1-P + ATP) using saturating amounts of substrates and activator as described by Boehlein *et al.* (2005).

Determining Heat Stability of AGPase

25 AGPase was purified as described above. AGPase was further diluted 1/100 (v/v) in 50mM HEPES, 5mM MgCl₂, 0.5mM EDTA and 0.5mg/mL BSA and heat treated at 42 or 53°C for various times, and then cooled on ice. The activity remaining after heat treatment was monitored in the forward and reverse direction by using saturating amounts of ATP, G-1-P and 3-PGA. The data were plotted as log of percentage of remaining
30 activity versus time of heat treatment. The inactivation constant $t_{1/2}$ was calculated from the formula $t_{1/2} = 0.693/(-2.3 \times \text{slope})$.

Qualitative Determination of the AGPase Purity

The purity of AGPase was monitored in the following way. Six µg of AGPase were diluted 1:1 in denaturing solution (100 mM Tris-Cl pH 6.8, 4% SDS, and 8mM DTT), heated at 95°C for 5min, electrophoresed on a 5% SDS polyacrylamide gel at 150V for 1h and visualized by staining with Coomassie Brilliant Blue (Laemmli, 1970).

Protein Blot Analysis of Crude Extracts

Samples were vacuum blotted onto a PVDF membrane (Biorad) by using Hybri-Dot blot apparatus (Life Technologies). The PVDF membrane had been pre-soaked in methanol for 5min and then in transfer buffer [20% (v/v) methanol, 0.303% (w/v) Tris and 1.44% (w/v) glycine] for 10min. The membrane was incubated with blocking buffer [0.8% (w/v) NaCl, 0.02% (w/v) KCl, 0.144% (w/v) Na₂HPO₄, 0.024% (w/v) KH₂PO₄, 5% (w/v) bovine serum albumin (BSA), and 0.05% (v/v) Tween-20] for 1h with constant shaking. The blot was incubated with blocking buffer containing 1:10000 (v/v) of monoclonal antibody against BT2 (kindly provided by Sue Boehlein) for 1h with shaking. Then, the blot was washed 3 x 10 min with washing buffer (blocking buffer –BSA) with constant shaking. The blot was then incubated with a 1:60000 dilution of goat anti-mouse secondary antibody conjugated with horseradish peroxidase (Pierce) for 45min. Finally the blot was washed 3 x 10 min. Proteins were visualized using an enhanced chemiluminescent substrate kit (Pierce).

3D Modeling

BT2 monomer structure was modeled after the potato small unit in the recently-published three dimensional structure of the potato tuber homotetrameric AGPase (RCSB Protein Data Bank #:1YP2). Homology modeling was done by using SWISS MODEL (Peitsch, 1995; Guex and Peitsch, 1997; Schwede *et al.* 2003; Kopp and Schwede, 2004; Arnold *et al.*, 2006). Amino acid 462 (Thr or Ile) contacting residues were determined by using Jmol, an open-source Java viewer for chemical structures in 3D, (<http://www.jmol.org/>).

Yeast Two Hybrid

Yeast transformations and a β-galactosidase assay were conducted as described by Greene and Hannah (1998b). The only modification was the use of pGBKT7 and pGADT7 as vectors for the bait and the prey respectively. pGBKT7-53 and pGADT7-T plasmids were used as a positive control.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

10 EXAMPLE 1

A mutant *Bt2* library was created by error-prone PCR. The mutational load was ~2 non-synonymous mutations per clone (Georgelis *et al.* 2007). The mutants were expressed in *E. coli* along with a wildtype *Sh2* gene. Approximately 50,000 colonies were screened for glycogen production. Ten dark staining colonies were picked. The two darkest staining *Bt2* mutants were sequenced. Both had the same non-synonymous mutation resulting in a change of amino acid 462 from threonine to isoleucine (TI). The threonine in that position is absolutely conserved among the higher plant small subunits (data not shown). BT2-TI/SH2 (BT2 comprising the TI mutation and complexed with SH2) produced more glycogen than did BT2/SH2 (Figure 1). Cells expressing BT2-TI and BT2, as homotetramers, did not produce detectable amounts of glycogen (Figure 1). This indicates that the amount of *E. coli*-synthesized glycogen depends exclusively on the complex of BT2-TI or BT2 with SH2.

A dot-blot of the crude extracts from cells expressing BT2/SH2 and BT2-TI/SH2 indicated that BT2-TI is found in higher amounts in *E. coli* (Figures 2A-2B). While AGPase activity levels of crude extracts from non-induced cells expressing BT2/SH2 and BT2-TI/SH2 were too low to detect, the partially purified extract from BT2-TI/SH2 had 20 times more activity than did the partially purified extract from BT2/SH2 (Figure 3). The possibility that BT2-TI/SH2 produced more protein and activity because of more efficient transcription/translation is unlikely since the codons ACA (T) to ATA (I) are used with the same frequency in *E. coli* (6.1 and 5.0% respectively) (Nakamura *et al.*, 2000). This suggests that the higher amount of protein and activity in BT2-TI/SH2 cells is due to increased stability of the AGPase.

To determine the kinetic properties and heat stability of BT2-TI/SH2 and decipher the cause of enhanced glycogen synthesis in *E. coli*, recombinant BT2-TI/SH2 and BT2/SH2 AGPases were purified (Figure 4). The kinetic properties of BT2-TI/SH2, as summarized in Table 3, show that the K_m for G-1-P and ATP, K_a for 3-PGA and K_i for Pi were indistinguishable from BT2/SH2. Surprisingly, the k_{cat} of BT2-TI is 30% lower than the k_{cat} of BT2/SH2. These kinetic properties then cannot account for the darker staining of BT2-TI/SH2 in *E. coli*. However, BT2-TI/SH2 is clearly more heat-stable than BT2/SH2 (Figures 5A-5B). These results strongly suggest that the high heat stability of BT2-TI/SH2 accounts for the enhanced amount of glycogen in *E. coli*.

MP is a small subunit variant that can lead to agronomic gain. Some of its features include increased activity in the absence of the activator 3-PGA, increased affinity for 3-PGA, decreased affinity for Pi (Table 3) and elevated heat stability compared to BT2/SH2 (Figure 3) (Cross *et al.*, 2004; Boehlein *et al.*, 2005). Since BT2-TI/SH2 was not as heat-stable as MP/SH2 (Figure 3), the amino acid change of TI was introduced into MP in an effort to further increase the heat stability of MP (MP-TI).

Cells expressing MP-TI/SH2 (MP having the TI mutation and complexed with SH2) produced the same amount of glycogen as cells expressing MP/SH2 (Figure 1). However, greater amounts of the MP-TI protein relative to BT2 were found in crude extracts of *E. coli* expressing the two proteins with SH2 (Figures 2A-2B). The activity of the crude extracts and the partially purified extracts from MP-TI/SH2 was 2-3 fold higher than from MP/SH2 (Figure 3). MP-TI/SH2, in its pure form (Figure 4), maintained the favorable kinetic properties of MP/SH2 (Table 3) except that its k_{cat} was reduced ~30% compared to MP/SH2. Additionally, MP-TI/SH2 exhibits greater heat stability than does MP/SH2 (Figures 6A-6B).

The crystal structure of maize endosperm AGPase has not been resolved. The only relevant structure is a potato tuber small subunit homotetramer (Jin *et al.*, 2005). The potato tuber small subunit shows 88% identity and 96% similarity to BT2. BT2 monomer structure was modeled after the resolved structure of the potato tuber small subunit (Figure 7A). The residue mutated in TI is part of a β -helix and it makes hydrophobic contact with two residues (Pro, Leu) of the N-terminus of the small subunit (Figure 7B). The amino acid change from Thr to Ile in TI shortens the distance from the Pro and Leu mentioned above (Figure 7C). It is tempting to speculate that the TI

mutation strengthens the hydrophobic interaction between the C- and the N- terminus of the small subunit and results in greater stability. Unlike MP, whose heat stability is attributed to residues at/near the subunit-subunit interfaces, TI may not directly affect subunit-subunit interactions since it is far from the subunit-subunit interfaces (Figure 7A).

5 To determine whether TI affects the strength of subunit-subunit interactions SH2 was used as a bait and BT2, TI, and MP were used as a prey in a yeast two-hybrid system (Y2H). A quantitative β -galactosidase assay indicated that, in contrast to MP, TI did not increase the strength of subunit-subunit interactions (Figure 8).

10 Finally, replacements of the original threonine by amino acids with shorter side chains such as serine, alanine and glycine did not affect the heat stability of AGPase even though they did result in 10-fold or more reduction in k_{cat} (data not shown). This indicates that the original threonine in position 462 is important for AGPase activity but not for heat stability.

15 EXAMPLE 2

The subject invention provides for agronomically important plant AGPase variants by using random mutagenesis and a heterologous bacterial expression system. BT2-TI was isolated as a small subunit variant that increased the amount of glycogen produced by *E. coli* cells when expressed along with SH2. Cells expressing BT2-TI/SH2
20 had 20-fold higher AGPase activity than cells expressing BT2/SH2. A dot-blot indicated that the crude protein extract from cells expressing BT2-TI/SH2 had more detectable BT2 protein compared to cells expressing BT2/SH2. This result could be attributed to more efficient transcription/ translation or to greater AGPase stability and/or solubility. As mentioned previously, a more efficient transcription/translation is unlikely based on
25 codon usage. On the other hand, it was showed that the purified form of BT2-TI/SH2 was significantly more heat-stable than the purified form of BT2/SH2. This may render the BT2-TI/SH2 complex less prone to proteolysis and/or aggregation compared to BT2/SH2 in *E. coli*.

The kinetic and allosteric properties of BT2-TI/SH2 were indistinguishable from
30 BT2/SH2 except for a 30% lower k_{cat} . The 20-fold increase in AGPase activity of BT2-TI/SH2 expressing cells is interpreted as a higher number of active AGPase molecules

compared to BT2/SH2 expressing cells. This also means that less than 5% of the potential AGPase molecules actually function in BT2/SH2.

It has been reported that the potato tuber small subunit can form a homotetramer that has significant activity when given extremely high amounts of the activator 3-PGA (Ballicora *et al.*, 1995). As shown herein, *E. coli* cells expressing BT2 and BT2-TI as homotetramers do not produce detectable amounts of glycogen. Hence, the increased amounts of glycogen observed in cells expressing BT2-TI/SH2 compared to cells expressing BT2/SH2 is due to the complex of BT2-TI with SH2 rather than the BT2-TI homotetramer.

Another small subunit variant that results in increased heat stability in a complex with SH2 is MP. The heat stability conferred by MP has been mapped to residues near or at the subunit-subunit interaction interfaces (Boehlein, Shaw, Stewart, and Hannah, unpublished data). In contrast, the amino acid change of BT2-TI is far from these interfaces. Structure modeling suggests that the TI change strengthens the intra-subunit hydrophobic interactions between the C- and the N-terminus. The results from Y2H support the idea that, in contrast to MP, TI does not strengthen the subunit-subunit interactions. However, the possibility that TI indirectly affects subunit-subunit interactions through a conformational change cannot be dismissed. It is possible that Y2H may not be sensitive enough to reveal a difference in subunit-subunit interactions between TI/SH2 and BT2/SH2 at the yeast growth temperature of 30°C.

A comparison of the heat stability of pure BT2-TI/SH2 and MP/SH2 indicated that BT2-TI/SH2 was not as heat-stable as MP/SH2. Additionally, MP/SH2 has several advantages not shared by BT2-TI/SH2, such as activity in the absence of the activator 3-PGA, a higher affinity for 3-PGA, a lower affinity for the inhibitor Pi and a higher k_{cat} compared to BT2/SH2. It was investigated whether the heat stability of MP/SH2 could be further improved by introducing the TI change into MP. The resulting variant, MP-TI, when expressed with SH2 in *E. coli*, yielded an equal amount of glycogen as MP/SH2. This could mean that either MP-TI/SH2 was not more heat-stable than MP/SH2 or that the production of ADP-glucose catalyzed by AGPase was not limiting in *E. coli* anymore. The latter interpretation may be favored since AGPase activity in crude and partially purified extracts of cells expressing MP-TI/SH2 was 2-3 fold higher than cells expressing MP/SH2. MP-TI/SH2 maintained all the kinetic and allosteric properties of MP/SH2

with the exception a 30% lower k_{cat} . Most importantly, MP-TI/SH2 was more heat-stable than MP/SH2. Two phases of heat stability in both MP-TI/SH2 and MP/SH2 were observed. These phases were probably a result of different states of AGPase. This biphasic mode of heat stability has been observed before and it is not specific to MP/SH2 or MP-TI (Boehlein *et al.* 2008). The first phase shows lower heat stability than does the second one. MP-TI/SH2 is more heat-stable in both phases compared to MP/SH2. However, what exactly the state of AGPase is in each phase remains enigmatic. The biphasic mode of heat stability was not observed in BT2/SH2 and BT2-TI/SH2 because the samples were not heated for long enough time to reach the second phase and they were heated at lower temperature than MP/SH2 and MP-TI/SH2 (42°C instead of 53°C).

Table 3 shows the kinetic properties of purified recombinant AGPase variants. The kinetic and allosteric properties of AGPase variants were determined in the forward direction. $k_{\text{cat}} (\text{s}^{-1})(\text{G-1-P})$ was estimated by varying the amount of G-1-P and keeping ATP at saturating amounts (1mM). $k_{\text{cat}} (\text{s}^{-1})(\text{ATP})$ was estimated by varying the amount of ATP and keeping G-1-P at saturating amounts (2mM). K_i 's are expressed as mean (95% confidence interval). All other values are expressed as mean $\pm$ standard deviation. The specific activity of AGPase in the absence of 3-PGA is expressed as a percentage of the specific activity in the presence of 10mM of 3-PGA (mean $\pm$ standard error).

Table 3.

	BT2/SH2	BT2-TI/SH2	MP/SH2	MP-TI/SH2
K_m G-1-P (mM)	0.050	0.040	0.079	0.059
	(± 0.008)	(± 0.006)	(± 0.007)	(± 0.005)
$k_{cat}(s^{-1})$ (G-1-P)	38.170	26.200	62.655	42.880
	(± 1.323)	(± 1.401)	(± 2.569)	(± 1.880)
K_m ATP (mM)	0.102	0.146	0.133	0.112
	(± 0.020)	(± 0.050)	(± 0.021)	(± 0.013)
$k_{cat}(s^{-1})$ (ATP)	43.321	29.112	69.337	49.031
	(± 1.554)	(± 1.030)	(± 3.276)	(± 1.903)
K_a 3-PGA (mM)	0.480	0.330	0.100	0.068
	(± 0.137)	(± 0.060)	(± 0.010)	(± 0.010)
K_i Pi(mM)	2.320	4.070	6.610	5.870
	(0.530,4.100)	(2.120,6.020)	(4.530,8.690)	(4.410,7.330)
$V_{max} - 3\text{-PGA} / V_{max} + 3\text{-PGA}$ (nmol/min/mg)				
BT2/SH2	BT2-TI/SH2	MP/SH2	MP-TI/SH2	
280/4000	134/2737	1856/6584	1101/4513	
(7.2 $\pm$ 3.2%)	(4.9 $\pm$ 1.5%)	(28.2 $\pm$ 3.1%)	(24.4 $\pm$ 5.3%)	

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 spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

REFERENCES

- U.S. Patent No. 5,034,322
U.S. Patent No. 5,106,739
5 U.S. Patent No. 5,589,610
U.S. Patent No. 5,589,618
U.S. Patent No. 5,625,136
U.S. Patent No. 5,639,948
U.S. Patent No. 5,650,557
10 U.S. Patent No. 5,661,017
U.S. Patent No. 5,872,216
U.S. Patent No. 6,069,300
U.S. Patent No. 6,184,438
U.S. Patent No. 6,403,863
15 U.S. Patent No. 6,455,760
U.S. Patent No. 6,462,185
U.S. Patent No. 6,696,623
U.S. Patent No. 6,809,235
U.S. Patent No. 6,969,783
20 U.S. Patent No. 7,173,165
U.S. Patent No. 7,312,378
U.S. Published Application No. 20030084486
U.S. Published Application No. 20030177536
U.S. Published Application No. 20040019934
25 U.S. Published Application No. 20040067506
U.S. Published Application No. 20040078841
U.S. Published Application No. 20040123349
International Published Application WO 2005/019425
International Published Application WO 99/58698
30 International Published Application WO 2003/0070901
International Published Application WO 98/22601
International Published Application WO 02/072784
EPO Patent Published Application No. EP1528104

- Altschul, S. F. *et al.* (1990) "Basic Local Alignment Search Tool" *J. Mol. Biol.* 215:402-410.
- 5 Altschul, S. F. *et al.* (1997) "Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs" *Nucl. Acids Res.* 25:3389-3402.
- Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. (2006) "The SWISS-MODEL Workspace: A web-based environment for protein structure homology modeling" *Bioinformatics* 22: 195-201.
- 10 Ballicora, M.A., Laughlin, M.J., Fu, Y., Okita, T.W., Barry, G.F. and Preiss, J. (1995) "Adenosine 5'-diphosphate-glucose pyrophosphorylase from potato tuber. Significance of the N-terminus of the small subunit for catalytic properties and heat stability" *Plant Physiol.* 109: 245-251.
- 15 Beltz, G. A., Jacobs, K. A., Eickbush, T. H., Cherbas, P. T., Kafatos, F. C. (1983) "Isolation of multigene families and determination of homologies by filter hybridization methods" *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285.
- Bhullar, S.S., and Jenner, C.F. (1985) "Differential responses to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat" *Aust. J. Plant Physiol.* 12: 363-375.
- 20 Boehlein, S.K., Sewell, A.K., Cross, J., Stewart, J.D., and Hannah, L.C. (2005) "Purification and characterization of adenosine diphosphate glucose pyrophosphorylase from maize/potato mosaics" *Plant Physiol.* 138: 1552-1562.
- Boehlein, S.K., Shaw, J.R., Stewart, J.D., and Hannah, L.C. (2008) "Heat Stability and Allosteric Properties of the Maize Endosperm ADP-Glucose Pyrophosphorylase Are Intimately Intertwined" *Plant Physiol.* 146: 289 - 299.
- 25 Chang, J. (1981) "Corn yield in relation to photoperiod, night temperature, and solar radiation" *Agricul. Metero.* 24: 253-262.
- Cheikn, N., and Jones, R. (1995) "Heat stress effects on sink activity of developing maize kernels grown *in vitro*" *Physiol. Plant.* 95: 59-66.
- 30 Christy, A.L., and Williamson, D.R. (1985) "Characteristics of CO₂ fixation and productivity of corn and soybeans" Pages 379-387 in P.W. Luden and J.E. Burris eds. Nitrogen Fixation and CO₂ Metabolism. Elsevier Science Publishing Co., New York.
- 35 Christy, A.L., Williamson, D.R., and Wideman, A.S. (1985) "Maize source development and activity. In 'Regulation of Carbon and Nitrogen Reduction and Utilization in Maize'" (Eds J.C. Shannon and C.D. Boyer.) pp 11-20. (American Society of Plant Physiologists: Rockville).
- 40 Clancy, M. and Hannah, L.C. (2002) "Splicing of the maize Sh1 first intron is essential for enhancement of gene expression, and a T-rich motif increases expression without affecting splicing" *Plant Physiol.* 130(2):918-29.

- Cross, J.M., Clancy, M., Shaw, J., Greene, T.W., Schmidt, R.R. Okita, T.W. and Hannah, L.C. (2004) "Both subunits of ADP-glucose pyrophosphorylase are regulatory" *Plant Physiol.* 135: 137-140.
- 5 Deng, Z., Roberts, D., Wang, X., and Kemp R.G. (1999) "Expression, characterization, and crystallization of the pyrophosphate-dependent phosphofructo-1-kinase of *Borrelia burgdorferi*" *Arch. Biochem. Biophys.* 371: 326-331.
- Duke, E., and Doehlert, D. (1996) "Effects of heat stress on enzyme activities and transcript levels in developing maize kernels grown in culture" *Environ. Exp. Botany* 36: 199-208.
- 10 Duncan, W.G., and Hesketh, J.D. (1968) "Net photosynthetic rates, relative leaf growth rates, and leaf numbers of 22 races of maize grown at eight temperatures" *Crop Science* 8: 670-674.
- Furtado, A. *et al.* (2002) "Tools for Use in the Genetic Engineering of Barley" *Proceedings of the 10th Australian Barley technical Symposium, Canberra, ACT, Australia.*
- 15 Georgelis, N., Braun, E.L., Shaw, J.R., and Hannah, L.C. (2007) "The two AGPase subunits evolve at different rates in angiosperm, yet they are equally sensitive to activity altering amino acid changes when expressed in bacteria" *Plant Cell* 19: 1458-1472.
- 20 Giroux, M.J., Shaw, J., Barry, G., Cobb, B.G., Greene, T.W., Okita, T.W., and Hannah, L.C. (1996) "A single mutation that increases maize seed weight" *Proc. Natl. Acad. Sci. USA* 93: 5824-5829.
- Good, X. *et al.* (1994) "Reduced ethylene synthesis by transgenic tomatoes expressing S-adenosylmethionine hydrolase" *Plant Molec. Biol.* 26:781-790.
- 25 Govons, S., Vinopal, R., Ingraham, J., and Preiss J. (1969) "Isolation of mutants of *Escherichia coli* B altered in their ability to synthesize glycogen" *J. Bacteriol.* 97: 970-972.
- Greene, T.W., and Hannah, L.C. (1998a) "Enhanced stability of maize endosperm ADP-glucose pyrophosphorylase is gained through mutants that alter subunit interactions" *Proc. Natl. Acad. Sci. USA* 95: 13342-13347.
- 30 Greene, T.W., and Hannah, L.C. (1998b) "Assembly of maize endosperm ADP-glucose pyrophosphorylase requires motifs located throughout the large and small subunit units" *Plant Cell* 10: 1295-1306.
- Greene, T.W., Kavakli, I.H., Kahn, M., and Okita, T.W. (1998) "Generation of up-regulated allosteric variants of potato ADP-glucose pyrophosphorylase by reversion genetics" *Proc. Natl. Acad. Sci. USA* 95: 10322-10327.
- 35 Guex, N., and Peitsch, M.C. (1997) "SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling" *Electrophoresis* 18: 2714-2723.
- Hall, T.A. (1999) "BioEdit, a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT" *Nucl. Acids Symp. Ser.* 41: 95-98.
- 40

- Hannah L.C., Shaw, J. R., Giroux, M., Reyss, A., Prioul, J.-L., Bae, J.-M. and Lee, J.-Y. (2001) "Maize Genes Encoding the Small Subunit of ADP-Glucose Pyrophosphorylase" *Plant Physiol.* 127:173-183.
- 5 Hannah, L.C., Tuschall, D., and Mans, R. (1980) "Multiple forms of maize endosperm ADP-glucose pyrophosphorylase and their control by Shrunken-2 and Brittle-2" *Genetics* 95: 961-970.
- Hannah, L.C., and Nelson, O.E., Jr. (1976) "Characterization of ADP-glucose pyrophosphorylase from shrunken-2 and brittle-2 mutants of maize" *Biochem. Genet.* 14: 547-560.
- 10 Hanson, R.S., and Phillips, J.A. (1981) "Chemical composition", p. 328-364. In P. Gerhardt, *et al.* (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
- Hofstra, G., and Hesketh, J.D. (1969) "Effects of temperature on the gas exchange of leaves in the light and dark" *Planta* 85: 228-237.
- 15 Hunter, R., Tollenaar, M., and Breuer, C. (1977) "Effects of photoperiod and temperature on vegetative and reproductive growth of maize (*Zea mays*) hybrid" *Can. J. Plant Sci.* 57: 1127-1133.
- Hwang, Y-S. *et al.* (2002) "Analysis of the Rice Endosperm-Specific Globulin Promoter in Transformed Rice Cells" *Plant Cell Rep.* 20:842-847.
- 20 Iglesias, A., Barry, G.F., Meyer, C., Bloksberg, L., Nakata, P., Greene, T., Laughlin M.J., Okita T.W., Kishore G.M., and Preiss, J. (1993) "Expression of the potato tuber ADP-glucose pyrophosphorylase in *Escherichia coli*" *J. Biol. Chem.* 268: 1081-1086.
- Jin, X., Ballicora, M.A., Preiss, J., and Geiger, J.H. (2005) "Crystal structure of potato tuber ADP-glucose pyrophosphorylase" *EMBO J.* 24: 694-704.
- 25 Jones, R., Ouattar, S., and Crookston, R. (1984) "Thermal environment during endosperm cell division and grain filling in maize: effects on kernel growth and development *in vitro*" *Crop Science* 24: 133-137.
- Karlin S. and Altschul, S. F. (1990) "Methods for Assessing the Statistical Significance of Molecular Sequence Features by Using General Scoring Schemes" *Proc. Natl. Acad. Sci. USA* 87:2264-2268.
- 30 Karlin S. and Altschul, S. F. (1993) "Applications and Statistics for Multiple High-Scoring Segments in Molecular Sequences" *Proc. Natl. Acad. Sci. USA* 90:5873-5877.
- 35 Kopp, J., and Schwede, T. (2004) "The SWISS-MODEL Repository of annotated three-dimensional protein structure homology models" *Nucleic Acids Research* 32: D230-D234.
- Laemmli, U.K. (1970) "Cleavage of structural proteins during the assembly of the head of bacteriophage T4" *Nature* 227: 680-685.
- 40 Lewin, B. (1985) *Genes II*, John Wiley & Sons, Inc., p. 96.
- Maniatis, T., E.F. Fritsch, J. Sambrook (1982) "Nuclease *Bal31*" *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

- Nakamura, Y., Gojobori, T., and Ikemura, T. (2000) "Codon usage tabulated from international DNA sequence databases: status for the year 2000" *Nucleic Acids Res.* 28: 292.
- Obana, Y., Omoto, D., Kato, C., Matsumoto, K., Nagai, Y., Kavakli, I.H., Hamada, S.,
5 Edwards, G.E., Okita, T.W., Matsui, H., and Ito, H. (2006) "Enhanced turnover of transitory starch by expression of up-regulated ADP-glucose pyrophosphorylase in *Arabidopsis thaliana*" *Plant Sci.* 170: 1-11.
- Peitsch, M.C. (1995) Protein modeling by E-mail Bio/Technology 13: 658-660.
- Peters, D.B., Pendleton, J.W., Hageman, R.H., and Brown, C.M. (1971) "Effect of night
10 air temperature on grain yield of corn, wheat and soybeans" *Agron. J.* 63: 809.
- Sakulsingharoja, C., Choi, S.B., Hwang, S.K., Edwards, G.E., Bork, J., Meyer, C.R., Preiss, J., and Okita, T.W. (2004) "Engineering starch biosynthesis for increasing rice seed weight: the role of the cytoplasmic ADP-glucose pyrophosphorylase" *Plant Sci.* 167: 1323-1333.
- 15 Schwede, T., Kopp, J., Guex, N., and Peitsch, M.C. (2003) "SWISS-MODEL: an automated protein homology-modeling server" *Nucleic Acids Res.* 31: 3381-3385.
- Singletary, G., Banisadr, R., and Keeling, P. (1993) "Decreased starch sythesis in heat stressed maize kernels results from reduced ADPG-pyrophosphorylase and starch synthase activities" *Plant Physiol. Suppl.* 102: 6.
- 20 Singletary, G., Banisadr, R., and Keeling, P. (1994) "Heat stress during grain filling in maize: effets of carbohydrate storage and metabolism" *Aust. J. Plant Physiol.* 21: 829-841.
- Smidansky, E.D., Clancy, M., Meyer, F.D., Lanning, S.P., Blake, N.K., Talbert, L.E., and Giroux, M.J. (2002) "Enhanced ADP-glucose pyrophosphorylase activity in wheat
25 endosperm increases seed yield" *Proc. Natl. Acad. Sci.* 99: 1724-1729.
- Smidansky, E.D., Martin, J.M., Hannah, L.C., Fischer, A.M., and Giroux, M.J. (2003) "Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase" *Planta* 216: 656-664.
- 30 Stark, D.M., Timmerman, K.P., Barry, G., Preiss, J., and Kishore, G.M. (1992) "Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase" *Science* 258: 287-292.
- Thompson, L. (1975) "Weather variability, climatic change and grain production" *Science* 188: 535-541.
- 35 Tollenaar, M., and Bruulsema, T. (1988) "Effects of temperature on rate and duration of kernel dry matter accumulation of maize" *Can. J. Plant Sci.* 68: 935-940.
- Tsai, C.Y., and Nelson, O.E. (1966) "Starch deficient maize mutants lacking adenosine diphosphate glucose pyrophosphorylase activity" *Science* 151: 341-343.
- Wallwork, M.A.B., Logue, S.J., MacLeod, L.C., and Jenner, C.F. (1998) "Effect of high
40 temperature during grain filling on starch synthesis in the developing barley grain" *Aust. J. Plant Physiol.* 25: 173-181.
- Wang, Z., Chen, X., Wang, J., Liu, T., Liu, Y., Zhao, L., and Wang, G. (2007) "Increasing maize seed weight by enhancing the cytoplasmic ADP-glucose

pyrophosphorylase activity in transgenic plants" *Plant Cell Tiss. Organ Cult.* 88: 83-92.

5 Wilhelm, E., Mullen, R., Keeling, P., and Singletary, G. (1999) "Heat stress during grain filling in maize: Effects on kernel growth and metabolism" *Crop Science* 39: 1733-1741.

Wu, C-L. *et al.* (1998) "Promoters of Rice Seed Storage Protein Genes Direct Endosperm-Specific Gene Expression in Transgenic Rice" *Plant and Cell Physiology*, 39(8):885-889.

10 Xu, D., McElroy, D., Thornburg, R. W., Wu, R. *et al.* (1993) "Systemic induction of a potato pin2 promoter by wounding, methyl jasmonate, and abscisic acid in transgenic rice plants" *Plant Molecular Biology* 22:573-588.

The claims defining the invention are as follows:

1. An isolated polynucleotide encoding a mutant plant AGPase small subunit protein, or a functional fragment of said protein, said protein comprising an amino acid mutation wherein the amino acid corresponding to the threonine amino acid at position 462 of wild type maize endosperm AGPase small subunit protein is replaced by an amino acid that confers increased heat stability when said mutant AGPase small subunit is expressed to form an AGPase enzyme.
2. The polynucleotide according to claim 1, wherein said replacement amino acid that confers increased heat stability is an isoleucine.
3. The polynucleotide according to claim 1 or 2, wherein said mutant AGPase small subunit is maize endosperm AGPase small subunit.
4. The polynucleotide according to claim 1, wherein said mutant plant AGPase small subunit protein encoded by said polynucleotide comprises the amino acid sequence shown in any of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, or a functional fragment thereof.
5. The polynucleotide according to claim 1, wherein said polynucleotide comprises the nucleotide sequence shown in any of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13, or a functional fragment thereof.
6. The polynucleotide according to claim 1, wherein said polynucleotide is provided in an expression construct.
7. A polynucleotide encoding a chimeric plant AGPase small subunit protein, or a functional fragment of said protein, wherein said chimeric AGPase protein comprises an N-terminus sequence from an N-terminus region of a plant AGPase small subunit from a first plant and a C-terminus sequence from a C-terminus region of a plant AGPase small subunit from a second plant, and said chimeric plant AGPase small subunit protein comprises an amino acid mutation wherein the amino acid corresponding to the threonine amino acid at position 462 of wild type maize endosperm AGPase small subunit protein is replaced by an amino acid that confers increased heat stability when said mutant AGPase small subunit is expressed to form an AGPase enzyme.
8. The polynucleotide according to claim 7, wherein said N-terminus sequence comprises the first 150 to 250 amino acids of the N-terminus region of said subunit of AGPase of said first plant and said C-terminus sequence comprises the terminal 300 residues or less of the C-terminus region of said subunit of AGPase of said second plant.

9. The polynucleotide according to claim 7 or claim 8, wherein said N-terminus region is from maize endosperm small subunit of AGPase.

10. The polynucleotide according to claim 7 or claim 8, wherein said C-terminus region is from potato tuber small subunit of AGPase.

11. The polynucleotide according to any one of claims 7 to 10, wherein said replacement amino acid that confers increased heat stability is an isoleucine.

12. The polynucleotide according to claim 7, wherein said plant AGPase small subunit protein encoded by said polynucleotide comprises the amino acid sequence shown in any of SEQ ID NO:4, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10, or a functional fragment thereof.

13. The polynucleotide according to claim 7, wherein said polynucleotide comprises the nucleotide sequence shown in any of SEQ ID NO:3, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16, or a functional fragment thereof.

14. The polynucleotide according to any one of claims 7 to 13, wherein said polynucleotide is provided in an expression construct.

15. The polynucleotide according to any one of claims 1, 2, 7 to 10 or 14, wherein said replacement amino acid that confers increased heat stability is a nonpolar amino acid.

16. The polynucleotide according to claim 15, wherein said replacement amino acid is selected from alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, or tryptophan.

17. An expression construct comprising the polynucleotide according to any one of claims 1 to 16.

18. A polynucleotide as defined in claim 1 or claim 7, or an expression construct as defined in claim 17, substantially as hereinbefore described with reference to any one of the examples.

19. An isolated polypeptide encoded by:

- a) a polynucleotide according to any one of claims 1 to 6 or any one of claims 15, 16 or 18 where dependent on any one of claims 1 to 6; or
- b) a polynucleotide according to any one of claims 7 to 14 or any one of claims 15, 16 or 18 where dependent on any one of claims 7 to 14.

20. A polypeptide as defined in claim 19, substantially as hereinbefore described

with reference to any one of the examples.

21. A transformed or transgenic plant or plant tissue or cell comprising:
 - a) a polynucleotide according to any one of claims 1 to 6 or any one of claims 15, 16 or 18 where dependent on any one of claims 1 to 6; or
 - b) a polynucleotide according to any one of claims 7 to 14 or any one of claims 15, 16 or 18 where dependent on any one of claims 7 to 14; or
 - c) the polynucleotides of both a) and b).
22. The plant or plant tissue or cell according to claim 21, wherein the plant or plant tissue or cell also expresses the maize large subunit of AGPase.
23. The plant or plant tissue or cell according to claim 21 or claim 22, wherein the plant or plant tissue or cell expresses a mutant large subunit of AGPase wherein said mutant large subunit comprises a mutation that confers increased heat stability and/or said mutant large subunit comprises a mutation that confers increased seed weight.
24. The plant or plant tissue or cell according to any one of claims 21 to 23, wherein said mutant large subunit comprises the Rev6 mutation.
25. The plant or plant tissue or cell according to any one of claims 21 to 24, wherein said plant or plant tissue or cell is monocotyledonous.
26. The plant or plant tissue or cell according to claim 25, wherein said monocotyledonous plant or plant tissue or cell is selected from the group consisting of rice, wheat, barley, oats, rye, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, turfgrasses, and millet.
27. The plant or plant tissue or cell according to any one of claims 21 to 26, wherein said plant is *Zea mays* or said plant tissue or cell is from *Zea mays*.
28. The plant or plant tissue or cell according to any one of claims 21 to 24, wherein said plant or plant tissue or cell is dicotyledonous.
29. The plant or plant tissue or cell according to claim 28, wherein said dicotyledonous plant or plant tissue or cell is selected from the group consisting of tomato, cucumber, squash, peas, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, beans, tobacco, potato, sweet potato, yams, cassava, radish, broccoli, spinach, cabbage, rape, apple trees, citrus (including oranges, mandarins, grapefruit, lemons, limes and the like), grape, cotton, sunflower, strawberry, lettuce, and hop.
30. The plant or plant tissue or cell according to any one of claims 21 to 29, wherein said plant tissue is a seed, scion, or rootstock.

31. An isolated mutant plant AGPase enzyme comprising one or more mutant polypeptides of claim 19.

32. A mutant plant AGPase enzyme as defined in claim 31, substantially as hereinbefore described with reference to any one of the examples.

33. A method of increasing resistance of a plant to heat stress conditions and/or increasing starch biosynthesis of a plant, said method comprising incorporating one or more polynucleotide into the genome of a plant and expressing the protein encoded by said polynucleotide, wherein said polynucleotide is or comprises:

a) any polynucleotide according to any one of claims 1 to 6 or any one of claims 15, 16 or 18 where dependent on any one of claims 1 to 6; and/or

b) any polynucleotide according to any one of claims 7 to 14 or any one of claims 15, 16 or 18 where dependent on any one of claims 7 to 14.

34. The method according to claim 33, wherein the plant also expresses the maize large subunit of AGPase.

35. The method according to claim 33 or claim 34, wherein the plant expresses a mutant large subunit of AGPase wherein said mutant large subunit comprises a mutation that confers increased heat stability and/or said mutant large subunit comprises a mutation that confers increased seed weight.

36. The method according to any one of claims 33 to 35, wherein said mutant large subunit comprises the Rev6 mutation.

37. The method according to any one of claims 33 to 36, wherein said plant is monocotyledonous.

38. The method according to claim 37, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, rye, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, turfgrasses, and millet.

39. The method according to any one of claims 33 to 38, wherein said plant is Zea mays.

40. The method according to any one of claims 33 to 36, wherein said plant is dicotyledonous.

41. The method according to claim 40, wherein said dicotyledonous plant is selected from the group consisting of tomato, cucumber, squash, peas, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, beans, tobacco, potato, sweet potato, yams, cassava, radish, broccoli, spinach, cabbage, rape, apple trees, citrus (including oranges,

mandarins, grapefruit, lemons, limes and the like), grape, cotton, sunflower, strawberry, lettuce, and hop.

42. A method for preparing a plant having an AGPase enzyme that provides for increased heat stability and/or increased starch biosynthesis in the plant relative to a plant having wild type AGPase enzyme, said method comprising introducing one or more polynucleotide into a plant cell and growing a plant from said plant cell, wherein said polynucleotide is or comprises:

a) any polynucleotide according to any one of claims 1 to 6 or any one of claims 15, 16 or 18 where dependent on any one of claims 1 to 6; and/or

b) any polynucleotide according to any one of claims 7 to 14 or any one of claims 15, 16 or 18 where dependent on any one of claims 7 to 14.

43. The method according to claim 42, wherein the plant also expresses the maize large subunit of AGPase.

44. The method according to claim 42, wherein the plant expresses a mutant large subunit of AGPase wherein said mutant large subunit comprises a mutation that confers increased heat stability and/or said mutant large subunit comprises a mutation that confers increased seed weight.

45. The method according to any one of claims 42 to 44, wherein said mutant large subunit comprises the Rev6 mutation.

46. The method according to any one of claims 42 to 45, wherein said plant is monocotyledonous.

47. The method according to claim 46, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, rye, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, turfgrasses, and millet.

48. The method according to any one of claims 42 to 47, wherein said plant is Zea mays.

49. The method according to any one of claims 42 to 45, wherein said plant is dicotyledonous.

50. The method according to claim 49, wherein said dicotyledonous plant is selected from the group consisting of tomato, cucumber, squash, peas, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, beans, tobacco, potato, sweet potato, yams, cassava, radish, broccoli, spinach, cabbage, rape, apple trees, citrus (including oranges, mandarins, grapefruit, lemons, limes and the like), grape, cotton, sunflower, strawberry,

2009234464 09 Jan 2015

lettuce, and hop.

University of Florida Research Foundation, Inc.
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

1/10

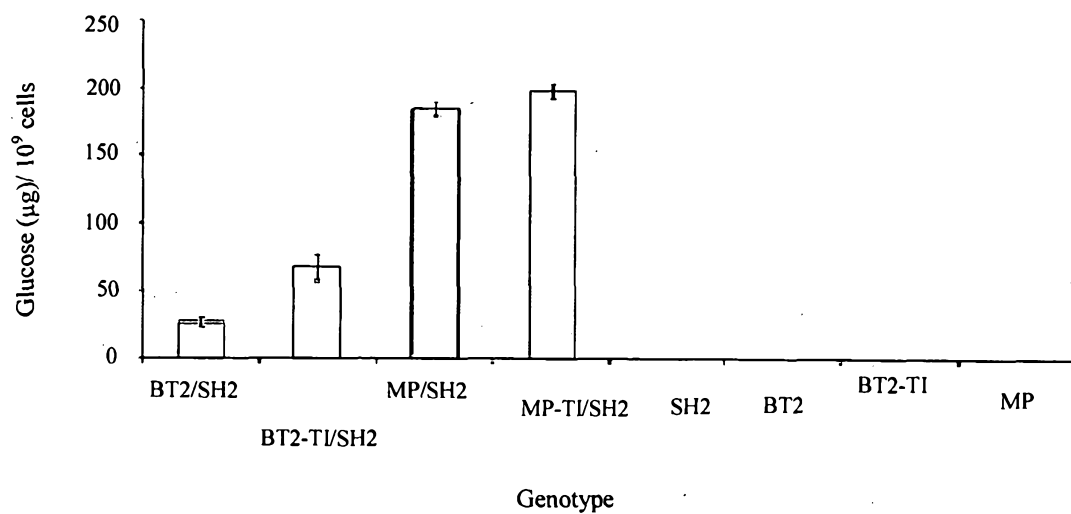


FIG. 1

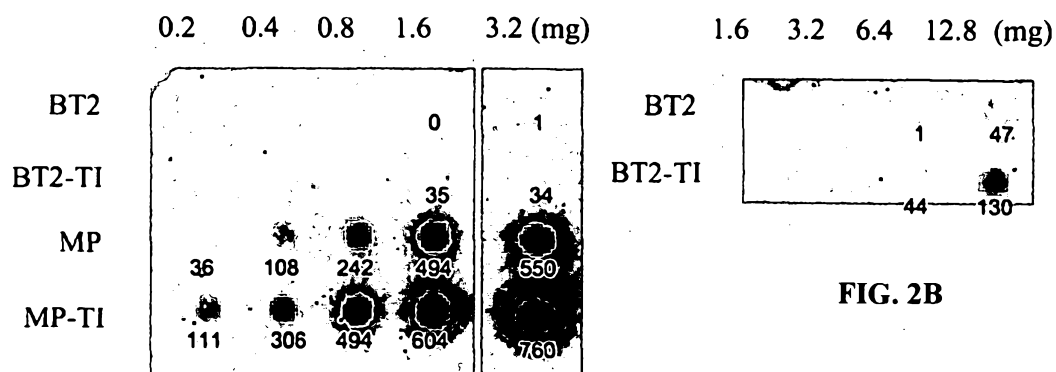


FIG. 2B

FIG. 2A

2/10

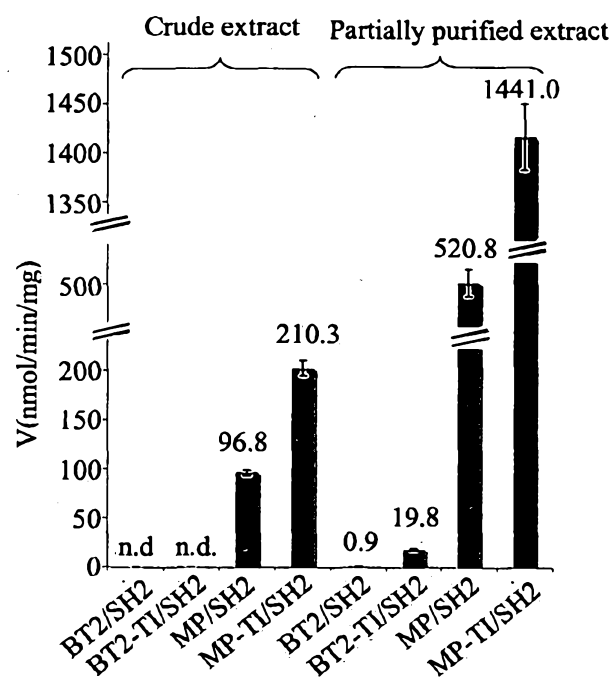


FIG. 3

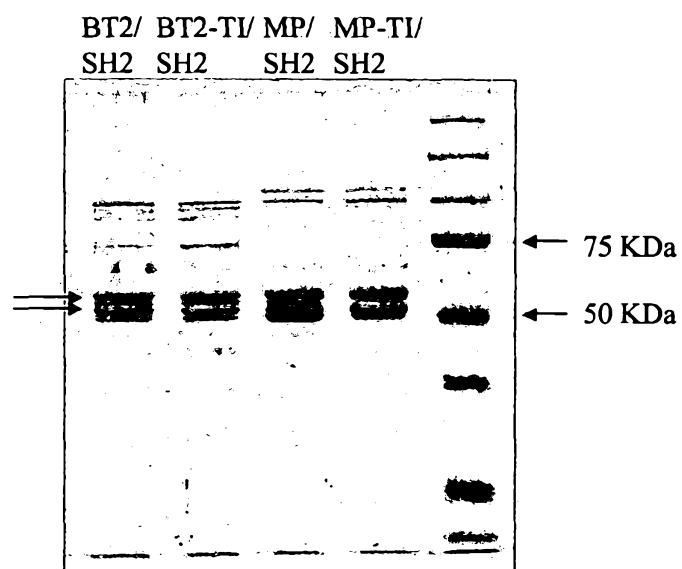


FIG. 4

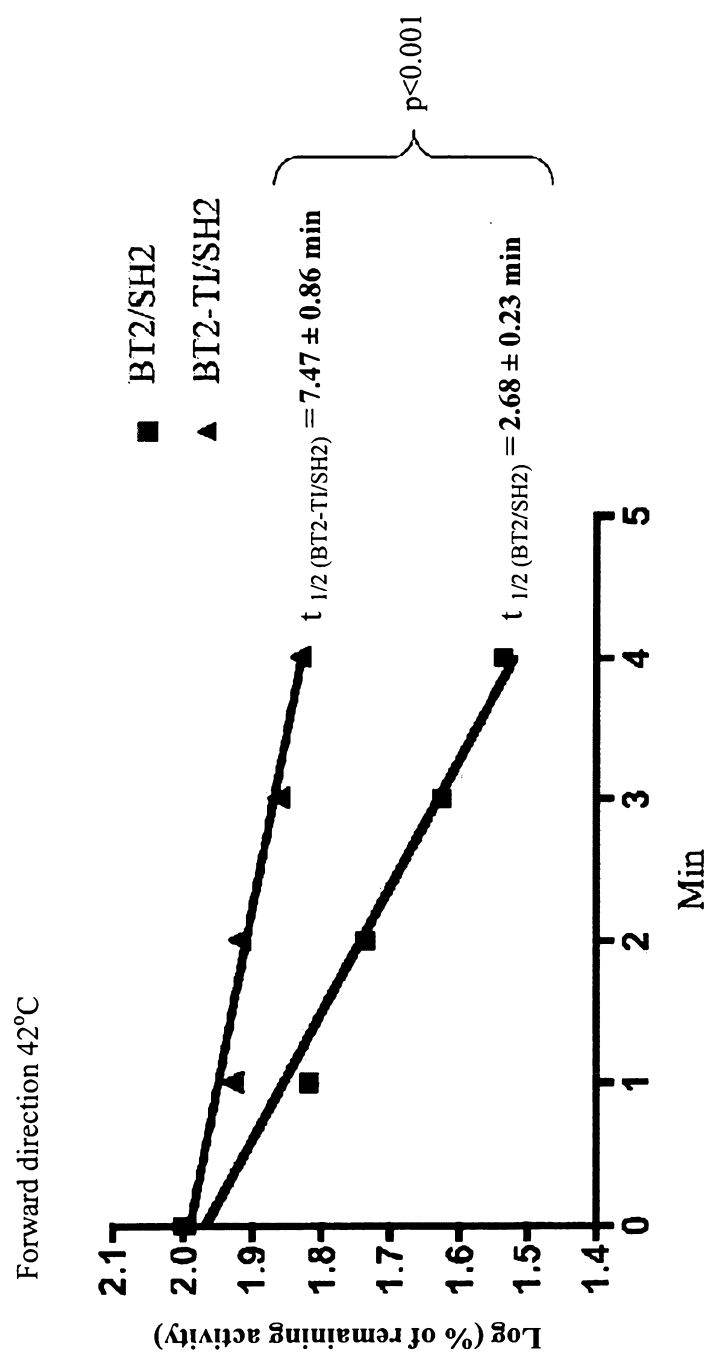


FIG. 5A

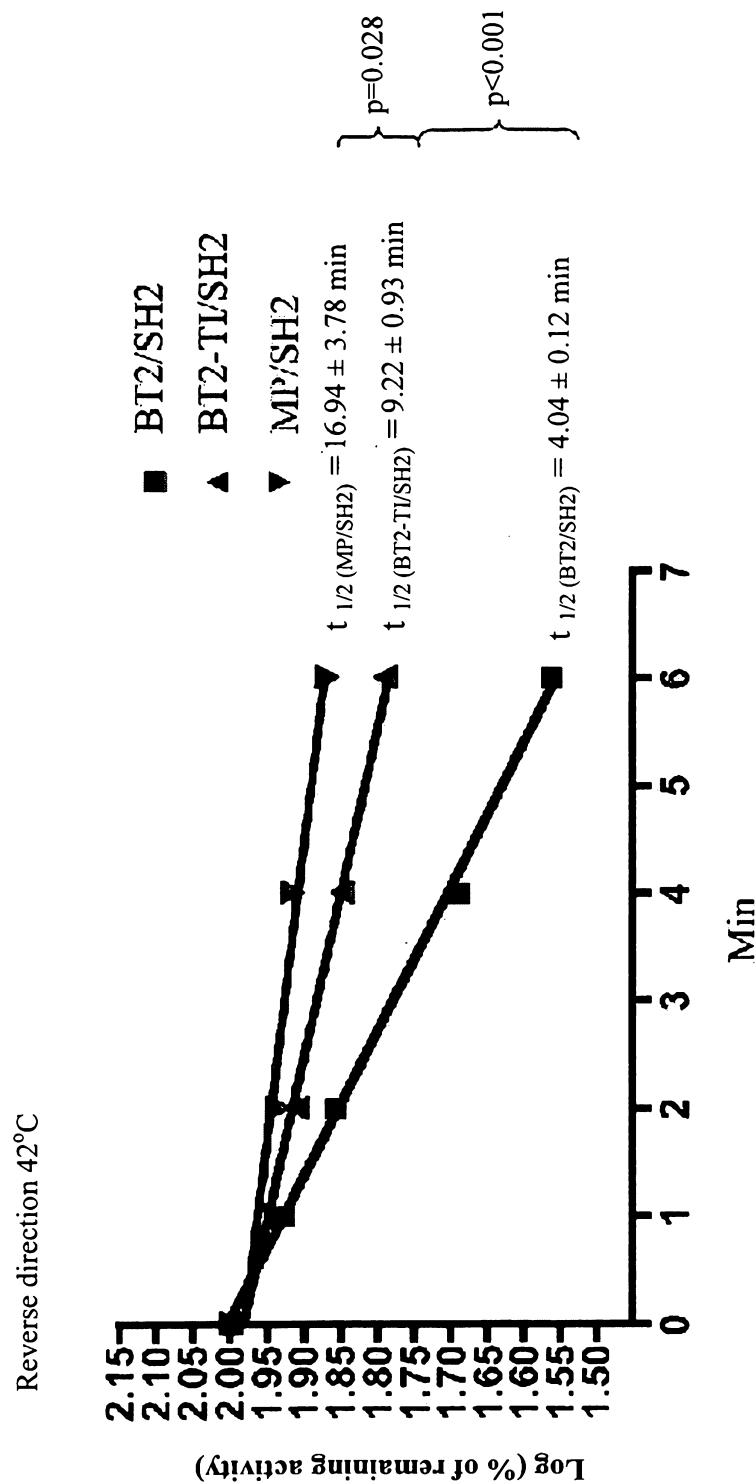


FIG. 5B

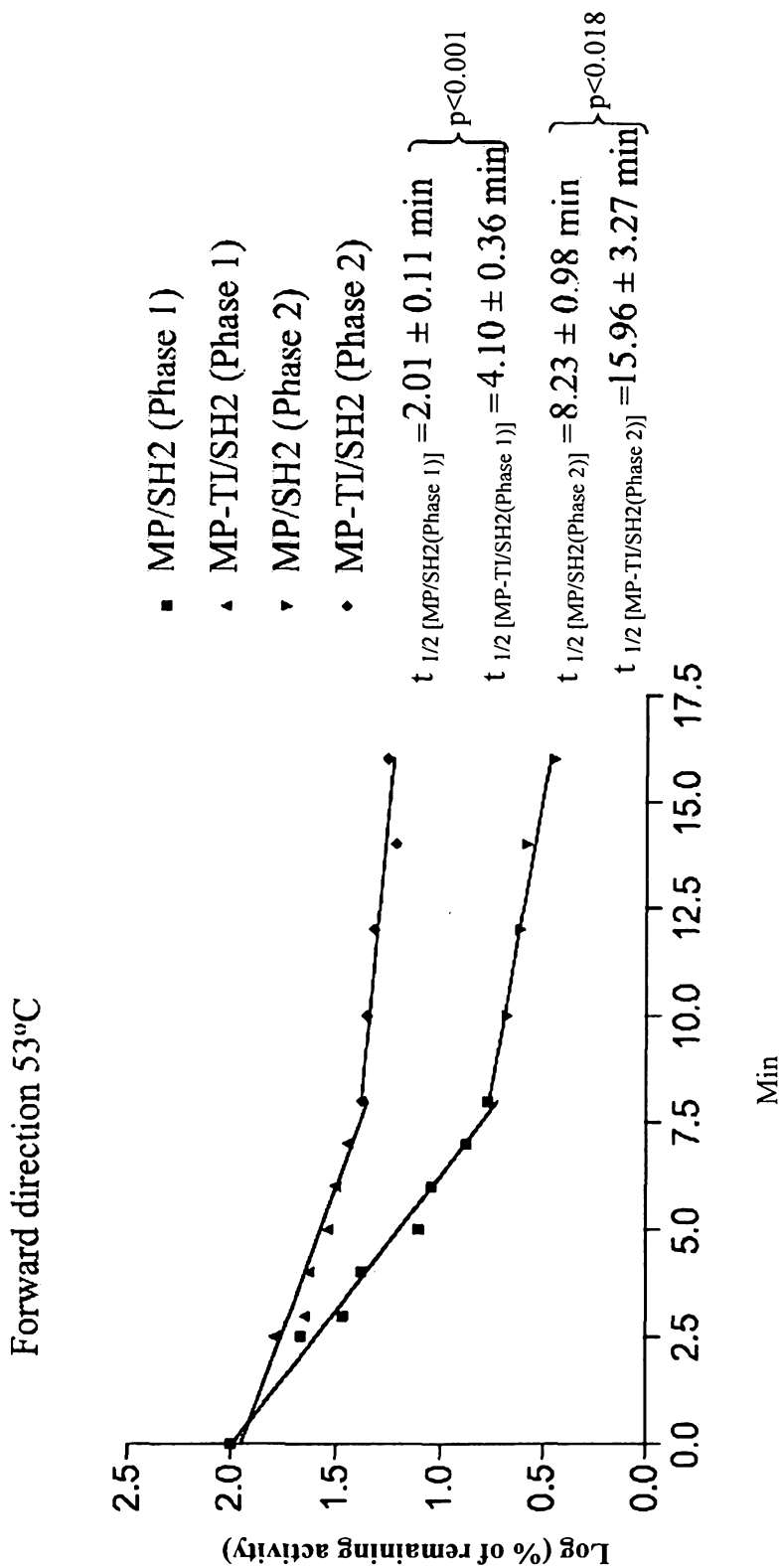


FIG. 6A

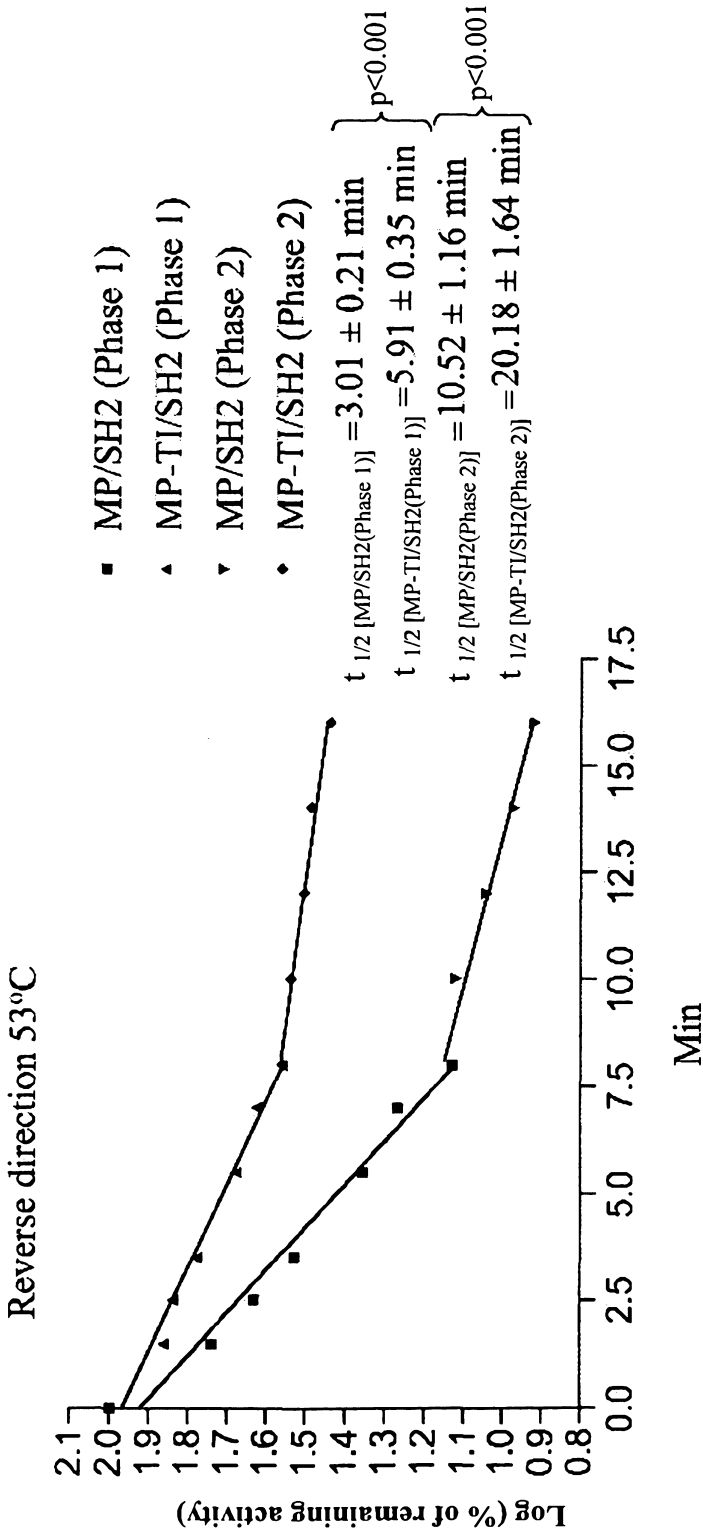
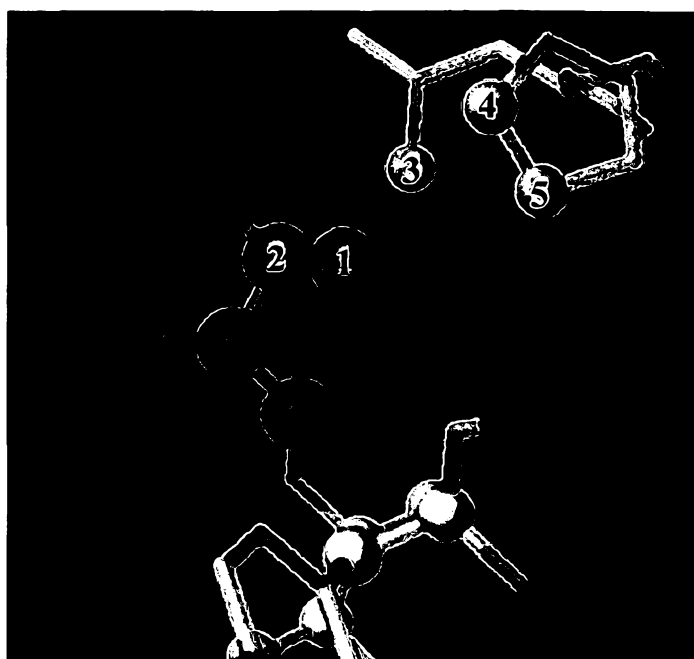


FIG. 6B

7/10



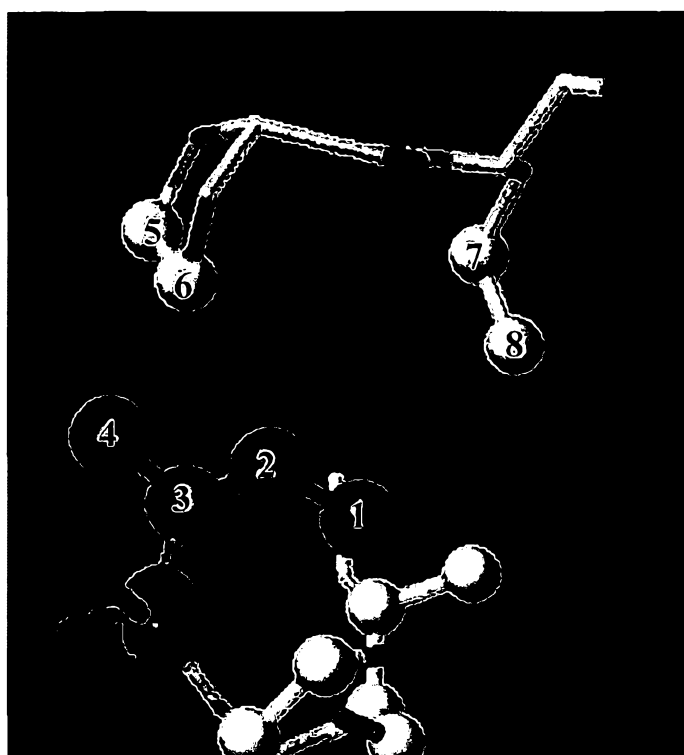
FIG. 7A



1-3: 3.90 Å
1-4: 3.92 Å
1-5: 3.81 Å
2-3: 5.23 Å
2-4: 4.16 Å
2-5: 4.31 Å

FIG. 7B

9/10



1-5: 5.25 Å
1-6: 5.02 Å
1-7: 4.33 Å
1-8: 3.39 Å
2-5: 3.90 Å
2-6: 3.76 Å
2-7: 4.53 Å
2-8: 4.00 Å
3-5: 4.30 Å
3-6: 4.44 Å
3-7: 5.60 Å
3-8: 5.25 Å
4-5: 3.27 Å
4-6: 3.61 Å
4-7: 6.02 Å
4-8: 5.96 Å

FIG. 7C

10/10

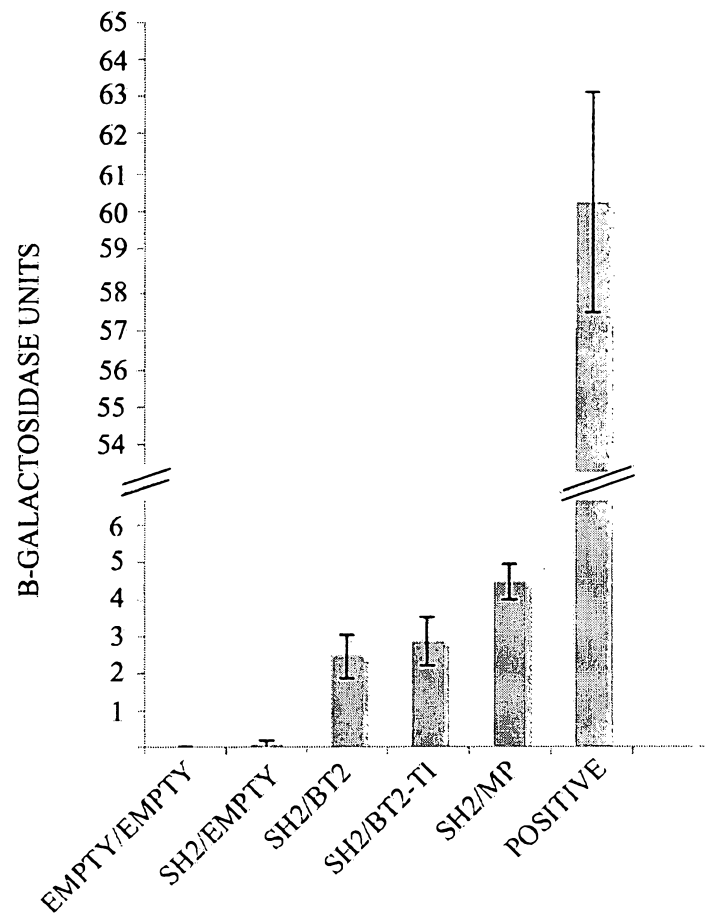


FIG. 8

SEQUENCE LISTING

<110> University of Florida Research Foundation, Inc.
Hannah, L. Curtis
Georgelis, Nikolaos

<120> Heat Resistant Plants and Methods and Materials for Making and
Using Same

<130> UF.698C1PCT

<150> 12/082,339
<151> 2008-04-09

<160> 26

<170> PatentIn version 3.5

<210> 1
<211> 1425
<212> DNA
<213> Artificial Sequence

<220>
<223> zea mays Bt2 gene

<220>
<221> misc_feature
<222> (1386)..(1386)
<223> h = a or c or t

<400> 1
atggacatgg ctttggcgtc taaagcctcc cctccgcat ggaatgccac cgccgccgag 60
cagccaattc caaagcgtga caaagccgct gcaaatgatt caacatacct caatcctcaa 120
gctcatgata gtgttcttgg aatcattctg ggaggtggtg ctgggactag attgtacccc 180
ttgacaaaga agcgtgccaa gcctgcagtg ccattgggtg ccaactatag actgattgat 240
attcctgtca gcaattgtct caacagcaac atatccaaga tctatgtgct aacgcaattt 300
aactctgctt cctcaaccg tcacctctca agagcctacg ggagcaacat tggagggtac 360
aagaatgaag ggtttgttga agtcttagct gcacagcaga gccagataa tccaaactgg 420
tttcagggtg ctgcagatgc tgtaaggcag tacttgggtg tgttgagga gcataatgtg 480
atggaatttc taattcttgc tggcgatcac ctgtaccgga tggactatga aaagttcatt 540

caggcacaca gagaacaaa tgctgatatt accgttgctg ccctaccgat ggatgagaaa 600
 cgtgcaactg catttggcct catgaaaatt gatgaagaag ggaggatcat tgagtttgct 660
 gagaaccga aaggagagca gttgaaagca atgatggtg acaccacat acttggcctt 720
 gatgacgtga gggcaaagga aatgccttat attgctagca tgggtatcta tgtttcagc 780
 aaagatgtaa tgcttcagct cctccgtgaa caatttcctg aagccaatga ctttggaagt 840
 gaggttattc caggtgcaac cagcattgga aagagggttc aggcttatct gtatgatggt 900
 tactgggaag atatcggtac cattgcggca ttataatg caaactggg aataaccaag 960
 aagccaatac cagatttcag ctctatgac cgtttgctc caatttatac acaacctga 1020
 cacctgccac ctcaaaggt tcttgatgct gatgtgacag acagtgttat tgggaagga 1080
 tgtgttatta aaaactgcaa gataaacat tctgtagttg gactccgac ttgcatact 1140
 gaaggtgcta tcatagagga cagtttacta atgggtgcgg actactatga gacagaagct 1200
 gataaaaaac tcctgccga aaaaggtggc attcctattg gtattgggaa aaattcatgc 1260
 atcaggagag caatcattga caagaatgct cgaattggag acaatgttaa gatactcaat 1320
 gctgacaatg ttcaagaagc tgcaatggag acagacgggt acttcatcaa aggtggaatt 1380
 gtcathgtga tcaaggatgc ttactccct agtgaacag ttata 1425

<210> 2

<211> 475

<212> PRT

<213> Artificial Sequence

<220>

<223> zea mays BT2 protein

<400> 2

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Thr Tyr Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly Ile
 35 40 45

Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys
 50 55 60

Arg Ala Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp
 65 70 75 80

Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val
 85 90 95

Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala
 100 105 110

Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val
 115 120 125

Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly Thr
 130 135 140

Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn Val
 145 150 155 160

Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr
 165 170 175

Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr Val
 180 185 190

Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met
 195 200 205

Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Lys
 210 215 220

Gly Glu Gln Leu Lys Ala Met Met Val Asp Thr Thr Ile Leu Gly Leu
 225 230 235 240

Asp Asp Val Arg Ala Lys Glu Met Pro Tyr Ile Ala Ser Met Gly Ile
 245 250 255

Tyr Val Phe Ser Lys Asp Val Met Leu Gln Leu Leu Arg Glu Gln Phe
 260 265 270

Pro Glu Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser
 275 280 285

Ile Gly Lys Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp
 290 295 300

Ile Gly Thr Ile Ala Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys
 305 310 315 320

Lys Pro Ile Pro Asp Phe Ser Phe Tyr Asp Arg Phe Ala Pro Ile Tyr
 325 330 335

Thr Gln Pro Arg His Leu Pro Pro Ser Lys Val Leu Asp Ala Asp Val
 340 345 350

Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile
 355 360 365

Asn His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile
 370 375 380

Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Glu Ala
 385 390 395 400

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

Lys Asn Ser Cys Ile Arg Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile
 420 425 430

Gly Asp Asn Val Lys Ile Leu Asn Ala Asp Asn Val Gln Glu Ala Ala
 435 440 445

Met Glu Thr Asp Gly Tyr Phe Ile Lys Gly Gly Ile Val Ile Val Ile
 450 455 460

Lys Asp Ala Leu Leu Pro Ser Gly Thr Val Ile
 465 470 475

<210> 3

<211> 1425

<212> DNA

<213> Artificial Sequence

<220>

<223> zea mays MP-TI nucleotide

<400> 3

atggacatgg ctttggcgtc taaagcctcc cctccgccat ggaatgccac cgccgccgag 60
 cagccaattc caaagcgtga caaagccgct gcaaatgatt caacatacct caatcctcaa 120
 gctcatgata gtgttcttgg aatcattctg ggaggtgggtg ctgggactag attgtacccc 180
 ttgacaaaga agcgtgccaa gcctgcagtg ccattgggtg ccaactatag actgattgat 240
 attcctgtca gcaattgtct caacagcaac atatccaaga tctatgtgct aacgcaattt 300
 aactctgctt ccccaaccg tcacctctca agagcctacg ggagcaacat tggagggtac 360
 aagaatgaag ggtttgttga agtcttagct gcacagcaga gccagataa tccaaactgg 420
 tttcagggtg ctgcagatgc tgtaaggcag tacttgtggt tgtttgagga gcataatgtg 480
 atggaatttc taattcttgc tggcgatcac ctgtaccgga tggactatga aaagttcatt 540
 caggcacaca gaaaacaaa tgctgatatt accgttgctg ccctaccgat ggatgagaag 600
 cgtgccactg catttggctc catgaagatt gacgaagaag gacgcattat tgaatttgca 660
 gagaaaccgc aaggagagca attgcaagca atgaaagtgg atactacat ttaggtctt 720
 gatgacaaga gagctaaaga aatgccttcc attgccagta tgggtatata tgcattagc 780
 aaagacgtga tgttaaacct acttcgtgac aagttccctg gggccaatga ttttgtagt 840
 gaagttattc ctggtgcaac ttacttggg atgagagtgc aagcttattt atatgatggg 900

tactgggaag atattggtac cattgaagct ttctacaatg ccaatttggg cattacaaaa 960
aagccggtgc cagattttag cttttacgac cgatcagccc caatctacac ccaacctcga 1020
tatctaccac catcaaaaat gcttgatgct gatgtcacag atagtgtcat tggatgaagg 1080
tgtgtgatca agaactgtaa gattcatcat tccgtggtg gactcagatc atgcatatca 1140
gagggagcaa ttatagaaga ctacttttg atgggggcag attactatga gactgatgct 1200
gacaggaagt tgttgctgc aaagggcagt gtccaattg gcatcggcaa gaattgtcac 1260
attaaagag ccattatcga caagaatgcc cgtatagggg acaatgtgaa gatcattaac 1320
aaagacaacg ttcaagaagc ggctagggaa acagatggat acttcatcaa gaggggatt 1380
gtcatcgtca tcaaggatgc ttgattcca agtggaatca tcatc 1425

<210> 4

<211> 475

<212> PRT

<213> Artificial Sequence

<220>

<223> zeamays MP-TI protein

<400> 4

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
20 25 30

Asp Ser Thr Tyr Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly Ile
35 40 45

Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys
50 55 60

Arg Ala Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp
65 70 75 80

Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val
85 90 95

Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala
100 105 110

Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val
115 120 125

Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly Thr
130 135 140

Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn Val
145 150 155 160

Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr
165 170 175

Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr Val
180 185 190

Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met
195 200 205

Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln
210 215 220

Gly Glu Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu
225 230 235 240

Asp Asp Lys Arg Ala Lys Glu Met Pro Phe Ile Ala Ser Met Gly Ile
245 250 255

Tyr Val Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe
260 265 270

Pro Gly Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser
275 280 285

Leu Gly Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp
 290 295 300

Ile Gly Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys
 305 310 315 320

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

Thr Gln Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val
 340 345 350

Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile
 355 360 365

His His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile
 370 375 380

Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala
 385 390 395 400

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

Lys Asn Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile
 420 425 430

Gly Asp Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala
 435 440 445

Arg Glu Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val Ile
 450 455 460

Lys Asp Ala Leu Ile Pro Ser Gly Ile Ile Ile
 465 470 475

<210> 5
 <211> 475
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> zea mays BT2 protein

<400> 5

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly Ile
 35 40 45

Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys
 50 55 60

Arg Ala Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp
 65 70 75 80

Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val
 85 90 95

Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala
 100 105 110

Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val
 115 120 125

Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly Thr
 130 135 140

Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn Val
 145 150 155 160

Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr
 165 170 175

Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr Val
 180 185 190

Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met
 195 200 205

Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Lys
 210 215 220

Gly Glu Gln Leu Lys Ala Met Met Val Asp Thr Thr Ile Leu Gly Leu
 225 230 235 240

Asp Asp Val Arg Ala Lys Glu Met Pro Tyr Ile Ala Ser Met Gly Ile
 245 250 255

Tyr Val Phe Ser Lys Asp Val Met Leu Gln Leu Leu Arg Glu Gln Phe
 260 265 270

Pro Glu Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser
 275 280 285

Ile Gly Lys Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp
 290 295 300

Ile Gly Thr Ile Ala Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys
 305 310 315 320

Lys Pro Ile Pro Asp Phe Ser Phe Tyr Asp Arg Phe Ala Pro Ile Tyr
 325 330 335

Thr Gln Pro Arg His Leu Pro Pro Ser Lys Val Leu Asp Ala Asp Val
 340 345 350

Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile
 355 360 365

Asn His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile
 370 375 380

Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Glu Ala
 385 390 395 400

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

Lys Asn Ser Cys Ile Arg Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile
 420 425 430

Gly Asp Asn Val Lys Ile Leu Asn Ala Asp Asn Val Gln Glu Ala Ala
 435 440 445

Met Glu Thr Asp Gly Tyr Phe Ile Lys Gly Gly Ile Val Ile Val Ile
 450 455 460

Lys Asp Ala Leu Leu Pro Ser Gly Thr Val Ile
 465 470 475

<210> 6

<211> 476

<212> PRT

<213> Artificial Sequence

<220>

<223> zea mays BT2 protein

<400> 6

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Gln Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly
 35 40 45

Ile Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys
 50 55 60

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

Asp Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr
 85 90 95

Val Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg
 100 105 110

Ala Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu
 115 120 125

Val Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly
 130 135 140

Thr Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn
 145 150 155 160

Val Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp
 165 170 175

Tyr Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr
 180 185 190

Val Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu
 195 200 205

Met Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro
 210 215 220

Lys Gly Glu Gln Leu Lys Ala Met Met Val Asp Thr Thr Ile Leu Gly
 225 230 235 240

Leu Asp Asp Val Arg Ala Lys Glu Met Pro Tyr Ile Ala Ser Met Gly
 245 250 255

Ile Tyr Val Phe Ser Lys Asp Val Met Leu Gln Leu Leu Arg Glu Gln
 260 265 270

Phe Pro Glu Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr
 275 280 285

Ser Ile Gly Lys Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu
 290 295 300

Asp Ile Gly Thr Ile Ala Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr
 305 310 315 320

Lys Lys Pro Ile Pro Asp Phe Ser Phe Tyr Asp Arg Phe Ala Pro Ile
 325 330 335

Tyr Thr Gln Pro Arg His Leu Pro Pro Ser Lys Val Leu Asp Ala Asp
 340 345 350

Val Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys
 355 360 365

Ile Asn His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala
 370 375 380

Ile Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Glu
 385 390 395 400

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

Gly Lys Asn Ser Cys Ile Arg Arg Ala Ile Ile Asp Lys Asn Ala Arg
 420 425 430

Ile Gly Asp Asn Val Lys Ile Leu Asn Ala Asp Asn Val Gln Glu Ala
 435 440 445

Ala Met Glu Thr Asp Gly Tyr Phe Ile Lys Gly Gly Ile Val Ile Val
 450 455 460

Ile Lys Asp Ala Leu Leu Pro Ser Gly Thr Val Ile
 465 470 475

<210> 7

<211> 476

<212> PRT

<213> Artificial Sequence

<220>

<223> zea mays BT2 protein

<400> 7

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Glu Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly
 35 40 45

Ile Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys
 50 55 60

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

Asp Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr
 85 90 95

Val Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg
 100 105 110

Ala Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu
 115 120 125

Val Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly
 130 135 140

Thr Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn
 145 150 155 160

Val Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp
 165 170 175

Tyr Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr
 180 185 190

Val Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu
 195 200 205

Met Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro
 210 215 220

Lys Gly Glu Gln Leu Lys Ala Met Met Val Asp Thr Thr Ile Leu Gly
 225 230 235 240

Leu Asp Asp Val Arg Ala Lys Glu Met Pro Tyr Ile Ala Ser Met Gly
 245 250 255

Ile Tyr Val Phe Ser Lys Asp Val Met Leu Gln Leu Leu Arg Glu Gln
 260 265 270

Phe Pro Glu Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr
 275 280 285

Ser Ile Gly Lys Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu
 290 295 300

Asp Ile Gly Thr Ile Ala Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr
 305 310 315 320

Lys Lys Pro Ile Pro Asp Phe Ser Phe Tyr Asp Arg Phe Ala Pro Ile
 325 330 335

Tyr Thr Gln Pro Arg His Leu Pro Pro Ser Lys Val Leu Asp Ala Asp
 340 345 350

Val Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys
 355 360 365

Ile Asn His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala
 370 375 380

Ile Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Glu
 385 390 395 400

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

Gly Lys Asn Ser Cys Ile Arg Arg Ala Ile Ile Asp Lys Asn Ala Arg
 420 425 430

Ile Gly Asp Asn Val Lys Ile Leu Asn Ala Asp Asn Val Gln Glu Ala
 435 440 445

Ala Met Glu Thr Asp Gly Tyr Phe Ile Lys Gly Gly Ile Val Ile Val
 450 455 460

Ile Lys Asp Ala Leu Leu Pro Ser Gly Thr Val Ile
 465 470 475

<210> 8

<211> 475

<212> PRT

<213> Artificial Sequence

<220>

<223> zea mays MP-TI protein

<400> 8

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly Ile
 35 40 45

Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys
 50 55 60

Arg Ala Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp
 65 70 75 80

Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val
 85 90 95

Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala
 100 105 110

Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val
 115 120 125

Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly Thr
 130 135 140

Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn Val
 145 150 155 160

Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr
 165 170 175

Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr Val
 180 185 190

Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met
 195 200 205

Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln
 210 215 220

Gly Glu Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu
 225 230 235 240

Asp Asp Lys Arg Ala Lys Glu Met Pro Phe Ile Ala Ser Met Gly Ile
 245 250 255

Tyr Val Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe
 260 265 270

Pro Gly Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser
 275 280 285

Leu Gly Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp
 290 295 300

Ile Gly Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys
 305 310 315 320

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

Thr Gln Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val
 340 345 350

Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile
 355 360 365

His His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile
 370 375 380

Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala
 385 390 395 400

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

Lys Asn Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile
 420 425 430

Gly Asp Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala
 435 440 445

Arg Glu Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val Ile
 450 455 460

Lys Asp Ala Leu Ile Pro Ser Gly Ile Ile Ile
 465 470 475

<210> 9

<211> 476

<212> PRT

<213> Artificial Sequence

<220>

<223> zea mays MP-TI protein

<400> 9

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Gln Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly
 35 40 45

Ile Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys
 50 55 60

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

Asp Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr
 85 90 95

Val Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg
 100 105 110

Ala Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu
 115 120 125

Val Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly
 130 135 140

Thr Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn
 145 150 155 160

Val Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp
 165 170 175

Tyr Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr
 180 185 190

Val Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu
 195 200 205

Met Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro
 210 215 220

Gln Gly Glu Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly
 225 230 235 240

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

Ile Tyr Val Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys
 260 265 270

Phe Pro Gly Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr
 275 280 285

Ser Leu Gly Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu
 290 295 300

Asp Ile Gly Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr
 305 310 315 320

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

Tyr Thr Gln Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp
 340 345 350

Val Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys
 355 360 365

Ile His His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala
 370 375 380

Ile Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp
 385 390 395 400

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

Gly Lys Asn Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg
 420 425 430

Ile Gly Asp Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala
 435 440 445

Ala Arg Glu Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val
 450 455 460

Ile Lys Asp Ala Leu Ile Pro Ser Gly Ile Ile Ile
 465 470 475

<210> 10
 <211> 476
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> zea mays MP-TI protein

<400> 10

Met Asp Met Ala Leu Ala Ser Lys Ala Ser Pro Pro Pro Trp Asn Ala
 1 5 10 15

Thr Ala Ala Glu Gln Pro Ile Pro Lys Arg Asp Lys Ala Ala Ala Asn
 20 25 30

Asp Ser Glu Thr Cys Leu Asn Pro Gln Ala His Asp Ser Val Leu Gly
 35 40 45

Ile Ile Leu Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys
 50 55 60

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

Asp Ile Pro Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr
 85 90 95

Val Leu Thr Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg
 100 105 110

Ala Tyr Gly Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu
 115 120 125

Val Leu Ala Ala Gln Gln Ser Pro Asp Asn Pro Asn Trp Phe Gln Gly
 130 135 140

Thr Ala Asp Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn
 145 150 155 160

Val Met Glu Phe Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp
 165 170 175

Tyr Glu Lys Phe Ile Gln Ala His Arg Glu Thr Asn Ala Asp Ile Thr
 180 185 190

Val Ala Ala Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu
 195 200 205

Met Lys Ile Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro
 210 215 220

Gln Gly Glu Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly
 225 230 235 240

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

Ile Tyr Val Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys
 260 265 270

Phe Pro Gly Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr
 275 280 285

Ser Leu Gly Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu
 290 295 300

Asp Ile Gly Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr
 305 310 315 320

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

Tyr Thr Gln Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp
 340 345 350

Val Thr Asp Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys
 355 360 365

Ile His His Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala
 370 375 380

Ile Ile Glu Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp
 385 390 395 400

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

Gly Lys Asn Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg
 420 425 430

Ile Gly Asp Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala
 435 440 445

Ala Arg Glu Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val
 450 455 460

Ile Lys Asp Ala Leu Ile Pro Ser Gly Ile Ile Ile
 465 470 475

<210> 11
 <211> 1425
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays Bt2 gene

<220>
 <221> misc_feature
 <222> (108)..(108)
 <223> y = t or c

<220>
 <221> misc_feature
 <222> (1386)..(1386)
 <223> h = a or c or t

<400> 11

atggacatgg ctttggcgtc taaagcctcc cctccgccat ggaatgccac cgccgccgag 60
 cagccaattc caaagcgtga caaagccgct gcaaatgatt caacatgycy caatcctcaa 120
 gctcatgata gtgttcttgg aatcattctg ggaggtgggtg ctgggactag attgtacccc 180
 ttgacaaaga agcgtgccaa gcctgcagtg ccattgggtg ccaactatag actgattgat 240
 attcctgtca gcaattgtct caacagcaac atatccaaga tctatgtgct aacgcaattt 300
 aactctgctt cctcaaccg tcacctctca agagcctacg ggagcaacat tggagggtac 360
 aagaatgaag ggtttgttga agtcttagct gcacagcaga gccagataa tccaaactgg 420
 tttcagggtg ctgcagatgc tgtaaggcag tacttgtggt tgtttgagga gcataatgtg 480
 atggaatttc taattcttgc tggcgatcac ctgtaccgga tggactatga aaagttcatt 540
 caggcacaca gagaacaaa tgctgatatt accgttgcct ccctaccgat ggatgagaaa 600
 cgtgcaactg catttggcct catgaaaatt gatgaagaag ggaggatcat tgagtttgct 660
 gagaaccga aaggagagca gttgaaagca atgatggtg acaccacat acttggcctt 720
 gatgacgtga gggcaaagga aatgccttat attgctagca tgggtatcta tgtttcagc 780
 aaagatgtaa tgcttcagct cctccgtgaa caatttcctg aagccaatga ctttgaagt 840
 gaggttattc caggtgcaac cagcattgga aagagggttc aggcttatct gtatgatggt 900
 tactgggaag atatcggtac cattgcggca ttataatg caaacttggg aataaccaag 960
 aagccaatac cagatttcag ctctatgac cgtttgctc caatttatac acaacctga 1020
 cacctgccac ctcaaaggt tcttgatgct gatgtgacag acagtgttat tgggaagga 1080
 tgtgttatta aaaactgcaa gataaacat tctgtagtgt gactccgac ttgcatact 1140
 gaaggtgcta tcatagagga cagtttacta atgggtgcgg actactatga gacagaagct 1200
 gataaaaaac tccttgccga aaaaggtggc attcctattg gtattgggaa aaattcatgc 1260
 atcaggagag caatcattga caagaatgct cgaattggag acaatgttaa gatactcaat 1320
 gctgacaatg ttcaagaagc tgcaatggag acagacgggt acttcatcaa aggtggaatt 1380
 gtcathgtga tcaaggatgc ttactccct agtgaacag ttata 1425

<211> 1428
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays Bt2 gene

<220>
 <221> misc_feature
 <222> (105)..(105)
 <223> r = g or a

<220>
 <221> misc_feature
 <222> (111)..(111)
 <223> y = t or c

<220>
 <221> misc_feature
 <222> (1389)..(1389)
 <223> h = a or c or t

<400> 12
 atggacatgg ctttggcgtc taaagcctcc cctccgccat ggaatgccac cgccgccgag 60
 cagccaattc caaagcgtga caaagccgct gcaaatgatt cacaracatg yctcaatcct 120
 caagctcatg atagtgttct tggaatcatt ctgggaggtg gtgctgggac tagattgtac 180
 cccttgacaa agaagcgtgc caagcctgca gtgccattgg gtgccaacta tagactgatt 240
 gatattcctg tcagcaattg tctcaacagc aacatatcca agatctatgt gctaacgcaa 300
 tttaactctg ctccctcaa ccgtcacctc tcaagagcct acgggagcaa cattggaggg 360
 tacaagaatg aagggtttgt tgaagtccta gctgcacagc agagcccaga taatccaaac 420
 tggtttcagg gtactgcaga tgctgtaagg cagtacttgt ggttgtttga ggagcataat 480
 gtgatggaat ttctaattct tgctggcgat cacctgtacc ggatggacta tgaaaagttc 540
 attcaggcac acagagaaac aaatgctgat attaccgttg ctgccctacc gatggatgag 600
 aaacgtgcaa ctgcatttgg cctcatgaaa attgatgaag aaggaggagg cattgagttt 660
 gctgagaaac cgaaaggaga gcagttgaaa gcaatgatgg ttgacaccac catacttggc 720
 cttgatgacg tgagggcaaa ggaaatgcct tatattgcta gcatgggtat ctatgttttc 780

agcaaagatg taatgcttca gctcctccgt gaacaatttc ctgaagccaa tgactttgga 840
 agtgagggtta ttccagggtgc aaccagcatt ggaaagaggg ttcaggctta tctgtatgat 900
 ggttactggg aagatatcgg taccattgcg gcattttata atgcaaactt gggaataacc 960
 aagaagccaa taccagattt cagcttctat gaccgttttg ctccaattta tacacaacct 1020
 cgacacctgc caccttcaaa ggttcttgat gctgatgtga cagacagtgt tattggtgaa 1080
 ggatgtgtta ttaaaaactg caagataaac cattctgtag ttggactccg atcttgcata 1140
 tctgaagggtg ctatcataga ggacagtta ctaatgggtg cggactacta tgagacagaa 1200
 gctgataaaa aactccttgc cgaaaaaggt ggcatccta ttggtattgg gaaaaattca 1260
 tgcatcagga gagcaatcat tgacaagaat gctcgaattg gagacaatgt taagatactc 1320
 aatgctgaca atgttcaaga agctgcaatg gagacagacg ggtacttcat caaaggtgga 1380
 attgtcathg tgatcaagga tgctttactc cctagtggaa cagttata 1428

<210> 13
 <211> 1428
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays Bt2 gene

<220>
 <221> misc_feature
 <222> (105)..(105)
 <223> r = g or a

<220>
 <221> misc_feature
 <222> (111)..(111)
 <223> y = t or c

<220>
 <221> misc_feature
 <222> (1389)..(1389)
 <223> h = a or c or t

<400> 13
 atggacatgg ctttggcgtc taaagcctcc cctccgcat ggaatgccac cgccgcccag 60

cagccaattc caaagcgtga caaagccgct gcaaatgatt cagaracatg yctcaatcct 120
 caagctcatg atagtgttct tggaatcatt ctgggaggtg gtgctgggac tagattgtac 180
 cccttgacaa agaagcgtgc caagcctgca gtgccattgg gtgccaacta tagactgatt 240
 gatattcctg tcagcaattg tctcaacagc aacatatcca agatctatgt gctaacgcaa 300
 ttaactctg ctccctcaa ccgtcacctc tcaagagcct acgggagcaa cattggaggg 360
 tacaagaatg aagggtttgt tgaagtctta gctgcacagc agagcccaga taatccaaac 420
 tggtttcagg gtactgcaga tgctgtaagg cagtacttgt gggtgtttga ggagcataat 480
 gtgatggaat ttctaattct tgctggcgat cacctgtacc ggatggacta tgaaaagttc 540
 attcaggcac acagagaaac aaatgctgat attaccgttg ctgccctacc gatggatgag 600
 aaacgtgcaa ctgcatttgg cctcatgaaa attgatgaag aaggaggat cattgagttt 660
 gctgagaaac cgaaaggaga gcagttgaaa gcaatgatgg ttgacaccac catacttggc 720
 cttgatgacg tgagggcaaa ggaaatgcct tatattgcta gcatgggtat ctatgttttc 780
 agcaaagatg taatgcttca gctcctccgt gaacaatttc ctgaagccaa tgactttgga 840
 agtgaggtta ttccaggtgc aaccagcatt ggaaagaggg ttcaggctta tctgtatgat 900
 gggtactggg aagatatcgg taccattgcg gcattttata atgcaaactt gggaataacc 960
 aagaagccaa taccagattt cagcttctat gaccgttttg ctccaattta tacacaacct 1020
 cgacacctgc caccttcaaa ggttcttgat gctgatgtga cagacagtgt tattggtgaa 1080
 ggatgtgtta ttaaaaactg caagataaac cattctgtag ttggactccg atcttgcata 1140
 tctgaaggtg ctatcataga ggacagtta ctaatgggtg cggactacta tgagacagaa 1200
 gctgataaaa aactccttgc cgaaaaaggt ggcatccta ttggtattgg gaaaaattca 1260
 tgcatcagga gagcaatcat tgacaagaat gctcgaattg gagacaatgt taagatactc 1320
 aatgctgaca atgttcaaga agctgcaatg gagacagacg ggtacttcat caaaggtgga 1380
 attgtcathg tgatcaagga tgctttactc ctagtggaa cagtata 1428

<210> 14

<211> 1425

<212> DNA

<213> Artificial Sequence

<220>

<223> zea mays MP-TI nucleotide

<220>

<221> misc_feature

<222> (108)..(108)

<223> y = t or c

<220>

<221> misc_feature

<222> (1386)..(1386)

<223> h = a or c or t

<400> 14

```

atggacatgg ctttggcgtc taaagcctcc cctccgccat ggaatgccac cgccgccgag    60
cagccaattc caaagcgtga caaagccgct gcaaatgatt caacatgyct caatcctcaa    120
gctcatgata gtgttcttgg aatcattctg ggaggtggtg ctgggactag attgtacccc    180
ttgacaaaga agcgtgccaa gcctgcagtg ccattgggtg ccaactatag actgattgat    240
attcctgtca gcaattgtct caacagcaac atatccaaga tctatgtgct aacgcaattt    300
aactctgctt ccctcaaccg tcacctctca agagcctacg ggagcaacat tggagggtac    360
aagaatgaag ggtttgttga agtcttagct gcacagcaga gccagataa tccaaactgg    420
tttcagggtg ctgcagatgc tgtaaggcag tacttgtggt tgtttgagga gcataatgtg    480
atggaatttc taattcttgc tggcgatcac ctgtaccgga tggactatga aaagttcatt    540
caggcacaca gagaacaaaa tgctgatatt accgttgctg ccctaccgat ggatgagaag    600
cgtgccactg catttggctc catgaagatt gacgaagaag gacgcattat tgaatttgca    660
gagaaaccgc aaggagagca attgcaagca atgaaagtgg atactacat ttaggtctt    720
gatgacaaga gagctaaaga aatgccttc attgccagta tgggtatata tgtcattagc    780
aaagacgtga tgttaaacct acttcgtgac aagttccctg gggccaatga ttttgtagt    840
gaagttattc ctggtgcaac ttcactggg atgagagtgc aagcttattt atatgatggg    900
tactgggaag atattggtac cattgaagct ttctacaatg ccaattggg cattacaaaa    960
aagccggtgc cagattttag cttttacgac cgatcagccc caatctacac ccaacctga   1020

```

tatctaccac catcaaaaat gcttgatgct gatgtcacag atagtgtcat tggatgaaggt 1080
 tgtgtgatca agaactgtaa gattcatcat tccgtgggtg gactcagatc atgcatatca 1140
 gagggagcaa ttatagaaga ctacttttg atgggggcag attactatga gactgatgct 1200
 gacaggaagt tgttggtgc aaagggcagt gtccaattg gcatcggcaa gaattgtcac 1260
 attaaaagag ccattatcga caagaatgcc cgtatagggg acaatgtgaa gatcattaac 1320
 aaagacaacg ttcaagaagc ggctagggaa acagatggat acttcatcaa gagggggatt 1380
 gtcathgtca tcaaggatgc ttgattcca agtggatca tcatc 1425

<210> 15
 <211> 1428
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays MP-TI nucleotide

<220>
 <221> misc_feature
 <222> (105)..(105)
 <223> r = g or a

<220>
 <221> misc_feature
 <222> (111)..(111)
 <223> y = t or c

<220>
 <221> misc_feature
 <222> (1389)..(1389)
 <223> h = a or c or t

<400> 15
 atggacatgg ctttgcgctc taaagcctcc cctcggccat ggaatgccac cgccgccgag 60
 cagccaattc caaagcgtga caaagccgct gcaaatgatt cacaracatg yctcaatcct 120
 caagctcatg atagtgttct tggaatcatt ctgggaggtg gtgctgggac tagattgtac 180
 cccttgacaa agaagcgtgc caagcctgca gtgccattgg gtgccaacta tagactgatt 240
 gatattcctg tcagcaattg tctcaacagc aacatatcca agatctatgt gctaacgcaa 300

tttactctg ctccctcaa ccgtcacctc tcaagagcct acgggagcaa cattggaggg 360
 tacaagaatg aagggtttgt tgaagtctta gctgcacagc agagcccaga taatccaaac 420
 tggtttcagg gtactgcaga tgctgtaagg cagtacttgt ggttgttga ggagcataat 480
 gtgatggaat ttctaattct tgctggcgat cacctgtacc ggatggacta tgaaaagttc 540
 attcaggcac acagagaaac aaatgctgat attaccgttg ctgccctacc gatggatgag 600
 aagcgtgcca ctgcatttgg tctcatgaag attgacgaag aaggacgcat tattgaattt 660
 gcagagaaac cgcaaggaga gcaattgcaa gcaatgaaag tggatactac cattttaggt 720
 cttgatgaca agagagctaa agaaatgcct tcattgcca gtatgggtat atatgtcatt 780
 agcaaagacg tgatgttaaa cctacttcgt gacaagttcc ctggggccaa tgattttggt 840
 agtgaagtta ttctggtgc aacttcactt gggatgagag tgcaagctta tttatatgat 900
 gggactctggg aagatattgg taccattgaa gctttctaca atgccaattt gggcattaca 960
 aaaaagccgg tgccagattt tagcttttac gaccgatcag cccaatcta cacccaacct 1020
 cgatatctac caccatcaaa aatgcttgat gctgatgtca cagatagtgt cattggtgaa 1080
 ggttgtgtga tcaagaactg taagattcat cattccgtgg ttggactcag atcatgcata 1140
 tcagaggag caattataga agactcactt ttgatggggg cagattacta tgagactgat 1200
 gctgacagga agttgttggc tgcaaagggc agtgtcccaa ttggcatcgg caagaattgt 1260
 cacattaaaa gagccattat cgacaagaat gcccgtagg gggacaatgt gaagatcatt 1320
 aacaaagaca acgttcaaga agcgggctagg gaaacagatg gatacttcat caagagtggg 1380
 attgtcathg tcatcaagga tgctttgatt ccaagtggaa tcatcatc 1428

<210> 16

<211> 1428

<212> DNA

<213> Artificial Sequence

<220>

<223> zea mays MP-TI nucleotide

<220>

<221> misc_feature

<222> (105)..(105)

<223> r = g or a

<220>

<221> misc_feature

<222> (111)..(111)

<223> y = t or c

<220>

<221> misc_feature

<222> (1389)..(1389)

<223> h = a or c or t

<400> 16

```

atggacatgg ctttggcgtc taaagcctcc cctccgcat ggaatgccac cgccgccgag    60

cagccaattc caaagcgtga caaagccgct gcaaatgatt cagaracatg yctcaatcct    120

caagctcatg atagtgttct tggaatcatt ctgggaggtg gtgctgggac tagattgtac    180

cccttgacaa agaagcgtgc caagcctgca gtgccattgg gtgccaacta tagactgatt    240

gatattcctg tcagcaattg tctcaacagc aacatatcca agatctatgt gctaacgcaa    300

ttaaactctg ctccctcaa ccgtcacctc tcaagagcct acgggagcaa cattggaggg    360

tacaagaatg aagggtttgt tgaagtctta gctgcacagc agagcccaga taatccaaac    420

tggttcagg gtactgcaga tgctgtaagg cagtacttgt gggtgttga ggagcataat    480

gtgatggaat ttctaattct tgctggcgat cacctgtacc ggatggacta tgaaaagttc    540

attcaggcac acagagaaac aaatgctgat attaccgttg ctgccctacc gatggatgag    600

aagcgtgcca ctgcatttgg tctcatgaag attgacgaag aaggacgcat tattgaattt    660

gcagagaaac cgcaaggaga gcaattgcaa gcaatgaaag tggatactac cattttaggt    720

cttgatgaca agagagctaa agaaatgcct tcattgcca gtatgggtat atatgtcatt    780

agcaaagacg tgatgttaaa cctacttcgt gacaagttcc ctggggccaa tgattttggt    840

agtgaagtta ttctgggtgc aacttcactt gggatgagag tgcaagctta tttatatgat    900

gggtactggg aagatattgg taccattgaa gctttctaca atgccaattt gggcattaca    960

aaaaagccgg tgccagattt tagcttttac gaccgatcag cccaatcta cacccaacct   1020

cgatatctac caccatcaaa aatgcttgat gctgatgtca cagatagtgt cattggtgaa   1080

```

gggtgtgtga tcaagaactg taagattcat cattccgtgg ttggactcag atcatgcata 1140
 tcagagggag caattataga agactcactt ttgatggggg cagattacta tgagactgat 1200
 gctgacagga agttgttggc tgcaaagggc agtgtcccaa ttggcatcgg caagaattgt 1260
 cacattaaaa gagccattat cgacaagaat gcccgtatag gggacaatgt gaagatcatt 1320
 aacaaagaca acgttcaaga agcggctagg gaaacagatg gatacttcat caagagtggg 1380
 attgtcathg tcatcaagga tgctttgatt ccaagtggaa tcatcatc 1428

<210> 17
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays oligonucleotide primer

<400> 17
 gaaggagata tatccatgg 19

<210> 18
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays oligonucleotide primer

<400> 18
 ggatccccgg gtaccgagct c 21

<210> 19
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays oligonucleotide primer

<400> 19
 gaaggagata tatccatgg 19

<210> 20
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> zea mays oligonucleotide primer

<400> 20
 gttgatatct gaattcgagc tc 22

<210> 21
 <211> 501
 <212> PRT
 <213> Hordeum vulgare subsp. vulgare

<400> 21

Met Met Ala Met Ala Thr Ala Met Ala Ala Thr Tyr Gly Ala Pro Ile
 1 5 10 15

Pro Ala Pro Ala Pro Ser Ala Ser Ser Pro Arg Arg Ala Ala Pro Asp
 20 25 30

Gly Gly Arg Arg Leu Arg Ala Val Ala Gly Arg Pro Arg Pro Leu Phe
 35 40 45

Ser Pro Arg Ala Val Ser Asp Ser Arg Asn Ser Gln Thr Cys Leu Asp
 50 55 60

Pro Asp Ala Ser Thr Ser Val Leu Gly Ile Ile Leu Gly Gly Gly Ala
 65 70 75 80

Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala Lys Pro Ala Val
 85 90 95

Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro Val Ser Asn Cys
 100 105 110

Leu Asn Ser Asn Val Ser Lys Ile Tyr Val Leu Thr Gln Phe Asn Ser
 115 120 125

Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Gly Asn Asn Ile Gly
 130 135 140

Gly Tyr Lys Asn Asp Gly Phe Val Glu Val Leu Ala Ala Gln Gln Ser
 145 150 155 160

Pro Glu Asn Pro Asn Trp Phe Gln Gly Thr Ala Asp Ala Val Arg Gln
 165 170 175

Tyr Leu Trp Leu Phe Glu Glu His Asn Val Met Glu Phe Leu Ile Leu
 180 185 190

Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Gln Lys Phe Ile Gln Ala
 195 200 205

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

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

Arg Ile Val Glu Phe Ser Glu Lys Pro Lys Gly Glu Lys Leu Lys Ala
 245 250 255

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

Glu Leu Pro Tyr Ile Ala Ser Met Gly Ile Tyr Val Phe Ser Lys Asp
 275 280 285

Ala Met Leu Arg Leu Leu Arg Asp Asn Phe Pro Ser Ala Asn Asp Phe
 290 295 300

Gly Ser Glu Val Ile Pro Gly Ala Thr Glu Ile Gly Met Arg Val Gln
 305 310 315 320

Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly Thr Ile Glu Ala

325

330

335

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

Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln Ser Arg Tyr Leu
 355 360 365

Pro Pro Ser Arg Val Leu Asp Ala Asp Val Thr Asp Ser Val Ile Gly
 370 375 380

Glu Gly Cys Val Ile Asn His Cys Lys Ile Asn His Ser Val Val Gly
 385 390 395 400

Leu Arg Ser Cys Ile Ser Glu Gly Ala Val Ile Glu Asp Ser Leu Leu
 405 410 415

Met Gly Ala Asp Tyr Tyr Glu Thr Glu Asn Asp Lys Lys Val Leu Ser
 420 425 430

Glu Thr Gly Gly Ile Pro Ile Gly Ile Gly Lys Asn Thr His Ile Lys
 435 440 445

Lys Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Glu Asn Val Lys Ile
 450 455 460

Ile Asn Val Asp Asp Ile Gln Glu Ala Ser Arg Glu Ser Asp Gly Tyr
 465 470 475 480

Phe Ile Lys Ser Gly Ile Val Ile Val Ile Lys Asp Ala Leu Ile Pro
 485 490 495

Ser Gly Thr Val Ile
 500

<210> 22

<211> 473

<212> PRT

<213> Triticum aestivum

<400> 22

Met Asp Val Pro Leu Ala Ser Lys Thr Phe Pro Ser Pro Ser Pro Ser
1 5 10 15

Lys Arg Glu Gln Cys Asn Val Asp Gly His Lys Ser Ser Ser Lys His
 20 25 30

Ala Asp Leu Asn Pro His Ala Asn Asp Ser Val Leu Gly Ile Ile Leu
 35 40 45

Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala
 50 55 60

Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro
65 70 75 80

Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu Thr
 85 90 95

Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Gly
 100 105 110

Ser Asn Ile Gly Gly Tyr Lys Asn Glu Gly Phe Val Glu Val Leu Ala
 115 120 125

Ala Gln Gln Ser Pro Asp Asn Pro Asp Trp Phe Gln Gly Thr Ala Asp
 130 135 140

Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Asn Val Met Glu
145 150 155 160

Tyr Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu Lys
 165 170 175

Phe Ile Gln Ala His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala Ala
 180 185 190

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

Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Lys Gly Glu
 210 215 220

Gln Leu Lys Ala Met Met Val Asp Thr Thr Ile Leu Gly Leu Asp Asp
 225 230 235 240

Ala Arg Ala Lys Glu Met Pro Tyr Ile Ala Ser Met Gly Ile Tyr Val
 245 250 255

Ile Ser Lys His Val Met Leu Gln Leu Leu Arg Glu Gln Phe Pro Gly
 260 265 270

Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser Thr Gly
 275 280 285

Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly
 290 295 300

Thr Ile Glu Ala Phe Tyr Asn Ala Asn Leu Gly Ile Thr Lys Lys Pro
 305 310 315 320

Ile Pro Asp Phe Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln
 325 330 335

Pro Arg His Leu Pro Pro Ser Lys Val Leu Asp Ala Asp Val Thr Asp
 340 345 350

Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His His
 355 360 365

Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile Glu
 370 375 380

Asp Thr Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Glu Ala Asp Lys
 385 390 395 400

Lys Leu Leu Ala Glu Lys Gly Gly Ile Pro Ile Gly Ile Gly Lys Asn
 405 410 415

Ser His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Asp
 420 425 430

Asn Val Met Ile Ile Asn Val Asp Asn Val Gln Glu Ala Ala Arg Glu
 435 440 445

Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val Ile Lys Asp
 450 455 460

Ala Leu Leu Pro Ser Gly Thr Val Ile
 465 470

<210> 23
 <211> 517
 <212> PRT
 <213> Sorghum bicolor

<220>
 <221> misc_feature
 <222> (383)..(389)
 <223> Xaa can be any naturally occurring amino acid

<400> 23

Met Ala Met Thr Ala Ile Ala Ser Pro Ser Ser Arg Thr Leu Ile Pro
 1 5 10 15

Pro Arg His His Gly Ala Ala Pro Ser Pro Ser Thr Ser Gly Asp Ser
 20 25 30

Ser Leu Arg Leu Leu Arg Ala His Pro Arg His Gly Arg Arg Ser Arg
 35 40 45

Gly Val Ser Val Ser Thr Pro Ala Ala Arg Ser Arg Pro Phe Val Phe

50 55 60

Ser Ser Arg Ala Val Ser Asp Ser Lys Ser Ser Gln Thr Cys Leu Asp
65 70 75 80

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

Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala Lys Pro Ala Val
100 105 110

Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro Val Ser Asn Cys
115 120 125

Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu Thr Gln Phe Asn Ser
130 135 140

Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Gly Ser Asn Ile Gly
145 150 155 160

Gly Tyr Lys Asn Glu Gly Phe Val Glu Val Leu Ala Ala Gln Gln Ser
165 170 175

Pro Asp Asn Pro Asn Trp Phe Gln Gly Thr Ala Asp Ala Val Arg Gln
180 185 190

Tyr Leu Trp Leu Phe Glu Glu His Asn Val Met Glu Phe Leu Ile Leu
195 200 205

Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu Lys Phe Ile Gln Ala
210 215 220

His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala Ala Leu Pro Met Asp
225 230 235 240

Glu Ala Arg Ala Thr Ala Phe Gly Leu Met Lys Ile Asp Glu Glu Gly
245 250 255

Arg Ile Ile Glu Phe Ala Glu Lys Pro Lys Gly Glu Gln Leu Lys Ala
 260 265 270

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

Glu Met Pro Tyr Ile Ala Ser Met Gly Ile Tyr Val Phe Ser Lys Asp
 290 295 300

Val Met Leu Gln Leu Leu Arg Glu Gln Phe Pro Gly Ala Asn Asp Phe
 305 310 315 320

Gly Ser Glu Val Ile Pro Gly Ala Thr Thr Ile Gly Lys Arg Val Gln
 325 330 335

Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly Thr Ile Thr Ala
 340 345 350

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

Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln Pro Arg Xaa Xaa
 370 375 380

Xaa Xaa Xaa Xaa Xaa Leu Asp Ala Asp Val Thr Asp Ser Val Ile Gly
 385 390 395 400

Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His His Ser Val Val Gly
 405 410 415

Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile Glu Asp Thr Leu Leu
 420 425 430

Met Gly Ala Asp Tyr Tyr Glu Thr Glu Ala Asp Lys Lys Leu Leu Ala
 435 440 445

Glu Asn Gly Gly Ile Pro Ile Gly Ile Gly Lys Asn Ser His Ile Arg

450 455 460

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

Leu Asn Ala Asp Asn Val Gln Glu Ala Ala Arg Glu Thr Asp Gly Tyr
 485 490 495

Phe Ile Lys Gly Gly Ile Val Ile Val Ile Lys Asp Ala Leu Leu Pro
 500 505 510

Ser Gly Thr Val Ile
 515

<210> 24

<211> 521

<212> PRT

<213> Solanum tuberosum

<400> 24

Met Ala Ala Ser Ile Gly Ala Leu Lys Ser Ser Pro Ser Ser Asn Asn
1 5 10 15

Cys Ile Asn Glu Arg Arg Asn Asp Ser Thr Arg Ala Val Ser Ser Arg
 20 25 30

Asn Leu Ser Phe Ser Ser Ser His Leu Ala Gly Asp Lys Leu Met Pro
 35 40 45

Val Ser Ser Leu Arg Ser Gln Gly Val Arg Phe Asn Val Arg Arg Ser
 50 55 60

Pro Met Ile Val Ser Pro Lys Ala Val Ser Asp Ser Gln Asn Ser Gln
65 70 75 80

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

Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala
 100 105 110

Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro
 115 120 125

Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu Thr
 130 135 140

Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Ala
 145 150 155 160

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

Ala Gln Gln Ser Pro Glu Asn Pro Asp Trp Phe Gln Gly Thr Ala Asp
 180 185 190

Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Thr Val Leu Glu
 195 200 205

Tyr Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu Lys
 210 215 220

Phe Ile Gln Ala His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala Ala
 225 230 235 240

Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met Lys Ile
 245 250 255

Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln Gly Glu
 260 265 270

Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu Asp Asp
 275 280 285

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

Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe Pro Gly
 305 310 315 320

Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser Leu Gly
 325 330 335

Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly
 340 345 350

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

Val Pro Asp Phe Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln
 370 375 380

Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val Thr Asp
 385 390 395 400

Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His His
 405 410 415

Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile Glu
 420 425 430

Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala Asp Arg
 435 440 445

Lys Leu Leu Ala Ala Lys Gly Ser Val Pro Ile Gly Ile Gly Lys Asn
 450 455 460

Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Asp
 465 470 475 480

Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala Arg Glu
 485 490 495

Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Ile Val Ile Lys Asp
 500 505 510

Ala Leu Ile Pro Ser Gly Ile Ile Ile
 515 520

<210> 25

<211> 500

<212> PRT

<213> Oryza sativa Japonica Group

<400> 25

Met Ala Met Met Ala Met Gly Ala Ala Ser Trp Ala Pro Ile Pro Ala
 1 5 10 15

Pro Ala Arg Ala Ala Ala Ala Phe Tyr Pro Gly Arg Asp Leu Ala Ala
 20 25 30

Ala Arg Arg Arg Arg Gly Ala Ala Ala Arg Arg Pro Phe Val Phe Thr
 35 40 45

Pro Arg Ala Val Ser Asp Ser Arg Ser Ser Gln Thr Cys Leu Asp Pro
 50 55 60

Asp Ala Ser Thr Ser Val Leu Gly Ile Ile Leu Gly Gly Gly Ala Gly
 65 70 75 80

Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala Lys Pro Ala Val Pro
 85 90 95

Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro Val Ser Asn Cys Leu
 100 105 110

Asn Ser Asn Val Ser Lys Ile Tyr Val Leu Thr Gln Phe Asn Ser Ala
 115 120 125

Ser Leu Asn Gly His Leu Ser Arg Ala Tyr Gly Asn Asn Ile Gly Gly
 130 135 140

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

Glu Asn Pro Asn Trp Phe Gln Gly Thr Ala Asp Ala Val Arg Gln Tyr
 165 170 175

Leu Trp Leu Phe Glu Glu His Asn Val Met Glu Phe Leu Ile Leu Ala
 180 185 190

Gly Asp His Leu Tyr Arg Met Asp Tyr Gln Lys Phe Ile Gln Ala His
 195 200 205

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

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

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

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

Leu Pro Tyr Ile Ala Ser Met Gly Ile Tyr Val Phe Ser Lys Asp Val
 275 280 285

Met Leu Lys Leu Leu Arg Gln Asn Phe Pro Ala Ala Asn Asp Phe Gly
 290 295 300

Ser Glu Val Ile Pro Gly Ala Thr Glu Ile Gly Met Arg Val Gln Ala
 305 310 315 320

Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly Thr Ile Glu Ala Phe
 325 330 335

Tyr Asn Ala Asn Leu Gly Ile Thr Lys Lys Pro Val Pro Asp Phe Ser

340 345 350

Phe Tyr Asp Arg Ser Ala Ala Ile Tyr Thr Gln Pro Arg Tyr Leu Pro
355 360 365

Pro Ser Lys Val Leu Asp Ala Asp Val Thr Asp Ser Val Ile Gly Glu
370 375 380

Gly Cys Val Ile Arg His Cys Thr Ile Asn His Ser Val Val Gly Leu
385 390 395 400

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

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

Thr Gly Gly Ile Pro Ile Gly Ile Gly Lys Asn Ala His Ile Arg Lys
435 440 445

Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Glu Asn Val Lys Ile Ile
450 455 460

Asn Val Asp Asn Ile Gln Glu Ala Ser Arg Glu Thr Asp Gly Tyr Phe
465 470 475 480

Ile Lys Ser Gly Ile Val Ile Val Ile Lys Asp Ala Leu Ile Pro Ser
485 490 495

Gly Thr Val Ile
500

<210> 26

<211> 521

<212> PRT

<213> Solanum tuberosum

<400> 26

Met Ala Ala Ser Ile Gly Ala Leu Lys Ser Ser Pro Ser Ser Asn Asn
 1 5 10 15

Cys Ile Asn Glu Arg Arg Asn Asp Ser Thr Arg Ala Val Ser Ser Arg
 20 25 30

Asn Leu Ser Phe Ser Ser Ser His Leu Ala Gly Asp Lys Leu Met Pro
 35 40 45

Val Ser Ser Leu Arg Ser Gln Gly Val Arg Phe Asn Val Arg Arg Ser
 50 55 60

Pro Met Ile Val Ser Pro Lys Ala Val Ser Asp Ser Gln Asn Ser Gln
 65 70 75 80

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

Gly Gly Gly Ala Gly Thr Arg Leu Tyr Pro Leu Thr Lys Lys Arg Ala
 100 105 110

Lys Pro Ala Val Pro Leu Gly Ala Asn Tyr Arg Leu Ile Asp Ile Pro
 115 120 125

Val Ser Asn Cys Leu Asn Ser Asn Ile Ser Lys Ile Tyr Val Leu Thr
 130 135 140

Gln Phe Asn Ser Ala Ser Leu Asn Arg His Leu Ser Arg Ala Tyr Ala
 145 150 155 160

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

Ala Gln Gln Ser Pro Glu Asn Pro Asp Trp Phe Gln Gly Thr Ala Asp
 180 185 190

Ala Val Arg Gln Tyr Leu Trp Leu Phe Glu Glu His Thr Val Leu Glu
 195 200 205

Tyr Leu Ile Leu Ala Gly Asp His Leu Tyr Arg Met Asp Tyr Glu Lys
 210 215 220

Phe Ile Gln Ala His Arg Glu Thr Asp Ala Asp Ile Thr Val Ala Ala
 225 230 235 240

Leu Pro Met Asp Glu Lys Arg Ala Thr Ala Phe Gly Leu Met Lys Ile
 245 250 255

Asp Glu Glu Gly Arg Ile Ile Glu Phe Ala Glu Lys Pro Gln Gly Glu
 260 265 270

Gln Leu Gln Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu Asp Asp
 275 280 285

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

Ile Ser Lys Asp Val Met Leu Asn Leu Leu Arg Asp Lys Phe Pro Gly
 305 310 315 320

Ala Asn Asp Phe Gly Ser Glu Val Ile Pro Gly Ala Thr Ser Leu Gly
 325 330 335

Met Arg Val Gln Ala Tyr Leu Tyr Asp Gly Tyr Trp Glu Asp Ile Gly
 340 345 350

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

Val Pro Asp Phe Ser Phe Tyr Asp Arg Ser Ala Pro Ile Tyr Thr Gln
 370 375 380

Pro Arg Tyr Leu Pro Pro Ser Lys Met Leu Asp Ala Asp Val Thr Asp
 385 390 395 400

Ser Val Ile Gly Glu Gly Cys Val Ile Lys Asn Cys Lys Ile His His
405 410 415

Ser Val Val Gly Leu Arg Ser Cys Ile Ser Glu Gly Ala Ile Ile Glu
420 425 430

Asp Ser Leu Leu Met Gly Ala Asp Tyr Tyr Glu Thr Asp Ala Asp Arg
435 440 445

Lys Leu Leu Ala Ala Lys Gly Ser Val Pro Ile Gly Ile Gly Lys Asn
450 455 460

Cys His Ile Lys Arg Ala Ile Ile Asp Lys Asn Ala Arg Ile Gly Asp
465 470 475 480

Asn Val Lys Ile Ile Asn Lys Asp Asn Val Gln Glu Ala Ala Arg Glu
485 490 495

Thr Asp Gly Tyr Phe Ile Lys Ser Gly Ile Val Thr Val Ile Lys Asp
500 505 510

Ala Leu Ile Pro Ser Gly Ile Ile Ile
515 520