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(54) Title: STARCHES WITH HIGH AMYLOSE CONTENT AND IMPROVED PROCESSING PROPERTIES

(57) **Abrégé/Abstract:**

The present invention relates in some embodiments to maize starches with an apparent amylose content between 35 wt. % and 90 wt. % and improved processing properties relative to conventional high-amylose maize starches. Furthermore, the present invention relates to maize meals and foodstuffs containing these maize starches or maize meals. In addition, the present invention relates to methods of production of said maize starches/maize meals and maize plants which synthesize these maize starches.

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The present invention relates in some embodiments to maize starches with an apparent amylose content between 35 wt. % and 90 wt. % and improved processing properties relative to conventional high-amylose maize starches. Furthermore, the present invention relates to maize meals and foodstuffs containing these maize

5 starches or maize meals. In addition, the present invention relates to methods of production of said maize starches/maize meals and maize plants which synthesize these maize starches.

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Starches with high amylose content and improved processing properties

The present invention relates to maize starches, embodiments of which have an apparent amylose content between 35 wt.% and 90 wt.% and which may have improved processing properties relative to conventional high-amylose maize starches. Furthermore, the present invention relates to maize meals and foodstuffs containing these maize starches or maize meals. In addition the present invention relates to methods of production of said maize starches/maize meals and maize plants which synthesize these maize starches.

Apart from oils, fats and proteins, polysaccharides are the main renewable raw materials from plants. Along with cellulose, starch occupies a key position among the polysaccharides, and is one of the most important storage substances in higher plants.

Furthermore, from the standpoint of nutritional physiology, starch is an important constituent of the human and animal diet. The structural characteristics of the starch present in foodstuffs can influence the functional (e.g. water binding capacity, swelling power), nutritional-physiological (e.g. digestibility, influence of the foodstuff on the glycemic index) or structure-forming (e.g. resistance to cutting, texture, stickiness, processability) properties of the most varied foodstuffs. Foodstuff compositions therefore often contain a starch with specified structural characteristics, which determine the desired properties of the foodstuff in question. The properties of foodstuffs containing starch-storing plant tissues (e.g. cereals, fruits, meals), can also be influenced by the starch contained in the plant tissues.

The polysaccharide starch is a polymer of chemically uniform basic building blocks, the glucose molecules. However, it is a very complex mixture of various molecular forms, which vary with respect to their degree of polymerization, the presence of branchings of the glucose chains and their chain lengths, and furthermore, may be modified, e.g. phosphorylated. Therefore starch is not a uniform raw material. In

particular we distinguish amylose, an essentially unbranched polymer of alpha-1,4-glycosidically linked glucose molecules, from amylopectin, which is a complex mixture of variously branched glucose chains. The branchings arise because of the presence of additional alpha-1,6-glycosidic linkages. In typical plants used for industrial starch production or as foodstuffs, such as maize, rice, wheat or potato, the starch that is synthesized consists of approx. 20% - 25% amylose and approx. 70% - 75% amylopectin.

Various methods are available for determining amylose content, and they can lead to different numerical values of the amylose content for one and the same starch. Many of these methods are based on the iodine-binding capacity of amylose, which can be determined potentiometrically (Banks & Greenwood, in W. Banks & C.T. Greenwood, *Starch and its components* (pp. 51-66), Edinburgh, Edinburgh University Press), amperometrically (Larson et al., *Analytical Chemistry* 25(5), (1953), 802-804) or spectrophotometrically (Morrison & Laignelet, *J. Cereal Sc.* 1, (1983), 9-20). The amylose content can also be determined calorimetrically by DSC (differential scanning calorimetry) measurements (Kugimiya & Donovan, *Journal of Food Science* 46, (1981), 765-770; Sievert & Holm, *Starch/Stärke* 45 (4), (1993), 136-139). It is also possible to determine the amylose content using SEC (size exclusion chromatography) chromatography of native or debranched starch. This method was recommended in particular for determining the amylose content of genetically engineered starches (Gérard et al., *Carbohydrate Polymers* 44, (2001), 19-27).

The functional, nutritional-physiological or structure-forming properties of starch, such as solubility, retrogradation behavior, water-binding capacity, film-forming properties, viscosity, gelatinization properties, freezing/thawing stability, acid resistance, gel strength, swelling power, digestibility, starch granule size of starches are influenced by, among other things, the structural characteristics of the starch, such as the amylose/amylopectin ratio, the molecular weight of the glucose polymers, the pattern of side chain distribution, the phosphate content, the lipid content or the protein content.

Using classical breeding methods, precise alteration of the structural/functional properties of the starch synthesized in plants is very difficult and is only possible for

selected structural characteristics. An alternative to breeding methods is the precise modification of starch-producing plants by the methods of genetic engineering. However, a precondition for this is the identification and characterization of the enzymes involved in starch synthesis and/or starch modification and their subsequent functional analysis in transgenic plants.

Maize mutants with an increased amylose content relative to wild-type maize plants are known and are designated as "amylose extenders" or "ae" for short. Amylose extender (ae) maize mutants were described for example in Vineyard and Bear (Maize Genet Coop Newsletter 26: 5 (1952), who describe the reference allele ae1-Ref, and in Moore and Creech (Genetics 70, (1972), 611-619), Garwood et al. (Cereal Chemistry 53(3), (1976), 355-364) and Hedman and Boyer (Biochemical Genetics 21 (11/12), (1983), 1217-1222).

Maize plants (cells) that have an "amylose extender mutation" show a mutation of the gene of the starch branching enzyme IIb from maize (abbreviation "BE IIb" or "SBE IIb"), which is also designated as amylose extender gene. This mutation leads to a decrease in SBE IIb enzyme activity in the endosperm of these maize plants compared to the BE IIb activity in the endosperm of wild-type maize plants. Preferably this mutation of BE IIb in maize plants (cells) has the effect that SBE IIb activity is no longer detectable (e.g. Fisher et al., Plant Physiol. 110, (1996), 611-619, in particular Fig. 4; Hedman and Boyer, Biochemical Genetics 21 (11/12), (1983), 1217-1222, in particular Table 1).

The "amylose extender mutation" is as a rule a recessive mutation of the amylose extender 1 locus.

Amylose extender mutants synthesize a high-amylose maize starch, which has an increased apparent amylose content compared with wild-type maize plants (plant cells), which as a rule is between 45 and 75 wt.%, depending on the genetic background, cultivation conditions and method of amylose determination.

Maize starches with the product designation Amylogel[®], Hylon[®] V and Hylon[®] VII are available commercially and as a rule have an apparent amylose content of approx. 50% or 70% (Shi et al., Journal of Cereal Science 27, (1998), 289-299).

In contrast to wild-type maize starches, these high-amylose maize starches have a

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greatly reduced swelling power (Senti and Russell, Tappi Vol. 43 No. 4, (1960), 343-349; Shi et al., Journal of Cereal Science 27, (1998), 289-299).

5 Aqueous suspensions of the high-amylose maize starches show no development of viscosity in viscosity analysis (e.g. RVA analysis) (Senti and Russell, Tappi Vol. 43 No. 4, (1960), 343-349). Native high-amylose maize starches so far only undergo gelatinization in a very energy-intensive and therefore cost-intensive method in an autoclave at elevated temperature and pressure, or they require subsequent chemical modification.

10 Based on the aforementioned properties, the high-amylose maize starches are very difficult to process, so that the range of applications in which the advantageous properties of the native high-amylose maize starches (e.g. very good film-forming properties, good gel-forming properties, use as resistant starch with prebiotic action) can be utilized is still very limited. Therefore there is a great demand for high-amylose maize starches with improved processing properties, such as increased
15 solubility in hot water, increased swelling power and capacity for viscosity development in aqueous suspensions under normal conditions.

Accordingly, one aim of the present invention is to make available high-amylose maize starches, or maize meals that contain these maize starches, which have improved processing properties compared with the known high-amylose maize
20 starches from amylose extender maize mutants, and to make maize plants (cells) available which synthesize said maize starches.

The present invention as claimed relates to a maize plant cell comprising a heterologous alpha-glucan, water dikinase (GWD) coding sequence and an amylose extender mutation.

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Figure 1 shows the RVA profile of Hylon VII® starch, Amylogel® and starch isolated from

A188 = wildtype maize plants,

AE = amylose extender maize mutant,

5 SC16 = transgenic GWD expressing maize plants,

SC16 x AE = crosses of transgenic GWD expressing maize plants with amylose extender maize mutants.

Figure 2 shows the detection of branching enzyme by means of activity gel analysis (zymogram) in single maize grains. Protein extracts were separated under native
10 conditions by polyacrylamide gel electrophoresis and then incubated with appropriate substrates. The resultant reaction products were stained with Lugol's solution.

WTA188 = wildtype maize plants,

AE = amylose extender maize mutant,

15 SC16 x AE = crosses of transgenic GWD expressing maize plants with amylose extender maize mutants.

Figure 3 Brabender viscosity profile of Hylon VII® starch and starch isolated from

A188 = wildtype maize plants (#1),

AE = amylose extender maize mutant (#11),

SC16 = transgenic GWD expressing maize plants (#2),

20 SC16 x AE = crosses of transgenic GWD expressing maize plants with amylose extender maize mutants (#8).

The present invention therefore relates to a maize starch, embodiments of which have an apparent amylose content between 35 wt.% and 90 wt.%, between 38 wt.% and 85 wt.%, between 40 wt.% and 80 wt.%, or between 42 wt.% and 75 wt.%, and
25 display certain viscosity properties in RVA analysis.

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In further embodiments the maize starch according to the invention have an apparent amylose content of at least 35 wt.%, at least 40 wt.%, or at least 42 wt.%.

In further embodiments the maize starch according to the invention have an apparent amylose content between 38 wt.% and 85 wt.%, between 40 wt.% and 80 wt.%, or
5 between 42 wt.% and 75 wt.%.

In embodiments of the present invention the maize starch according to the invention display, in an aqueous suspension in RVA analysis, an increase in viscosity of more than 60 centipoise (cP), more than 100, or more than 200 centipoise (cP).

Methods of determination of the amylose content are known by a person skilled in the
10 art. In connection with the present invention, amylose content means the content of apparent amylose as a percentage by weight relative to the amount of starch. The apparent amylose content of a starch/of a meal is determined in connection with the present invention preferably by the method described below (see "General methods, Determination of the content of apparent amylose").

15 In an especially preferred embodiment, the maize starch according to the invention is a native maize starch.

The term "native maize starch" means, in connection with the present invention, that the maize starch is isolated by methods known by a person skilled in the art from maize plants according to the invention, from starch-storing parts of the maize plants
20 according to the invention or from propagating material according to the invention. After isolation, no additional chemical derivatization (e.g. acetylation, oxidation, crosslinking etc.) takes place for the native maize starches according to the invention.

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The term "RVA analysis" means, in connection with the present invention, that the viscosity properties of the starches (according to the invention) are determined by means of a "Rapid Visco Analyzer" (=RVA). RVA instruments are for example made and marketed by the company Newport Scientific Pty Ltd, Investment Support Group, 5 Warriewood, NSW 2102, Australia. RVA analysis is the standard method of analysis for investigation of the gelatinization properties of starch.

In connection with the present invention, RVA analysis of the starches according to the invention is preferably carried out according to AACC Standard Method No. 61-02, "Determination of the Pasting Properties of Rice with the Rapid Visco 10 Analyzer" (Final Approval October 15, 1997; Reapproval November 3, 1999; Approved Methods of the American Association of Cereal Chemists (AACC) - 10th edition - Including 2001, 2002 and 2003 Supplements, American Association of Cereal Chemists (2003), St Paul MN, USA). Instead of the rice flour samples described in this AACC method, in connection with the present invention the maize 15 starches according to the invention are analyzed. In connection with the present invention, preferably the temperature and shear profile described in AACC method 61-02 is employed, which is also described later (see "General methods").

In an embodiment of the present invention, the changes in the viscosity properties in RVA analysis according to AACC method 61-02 relate to a 10% (w/v) aqueous 20 suspension of the starch according to the invention in water.

Using RVA analysis, various properties can be determined, such as the pasting temperature, the peak viscosity, the final viscosity and the setback.

In some embodiments, the increase in viscosity of an aqueous suspension of the maize starch according to the invention in RVA analysis is more than 25 250 centipoise (cP), more than 500, or more than 1000 centipoise (cP) compared to the viscosity in centipoise (cP) at the start of the RVA analysis.

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In some embodiments the increase in viscosity of an aqueous suspension of the maize starch according to the invention in RVA analysis is between 200 and 2000 centipoise (cP), between 1000 and 1800 centipoise (cP), or between 1300 and 1700 centipoise (cP).

- 5 In some embodiments, the maize starch according to the invention displays, in RVA analysis, a measurable pasting temperature of at least 75°C, at least 80°C, or at least 85°C.

In some embodiments the maize starch according to the invention displays, in RVA analysis, a pasting temperature between 78°C and 95°C, between 80°C and 93°C, or
10 between 82°C and 91°C.

In contrast to conventional native high-amylose starches, which display little if any gelatinization in RVA analysis under normal pressure, the starches according to the invention have the advantage that despite the increased amylose content they gelatinize under standard conditions. As a result the processability of the starch
15 according to the invention may be greatly improved compared with other high-amylose starches. Therefore gelatinization of the starches according to the invention does not require the same increase in temperature or pressure as required for conventional native high-amylose starches; this may lead to energy savings and therefore cost savings.

20 The term "pasting temperature" (RVA PT = RVA Pasting Temperature) means, in connection with the present invention, that the measured value for the start of viscosity development according to RVA analyses is the temperature at which the viscosity curve during the heating process leaves the baseline and at which, in a time interval of 0.5 min, the viscosity changes by more than 50 centipoise (cP).

25 In further embodiments the maize starches according to the invention have a peak viscosity between 1200 and 2000 centipoise (cP), between 1400 and 1900 centipoise (cP), or between 1500 and 1850 centipoise (cP).

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In further embodiments the maize starches according to the invention have a peak viscosity of at least 1200, or at least 1500 centipoise (cP).

In further embodiments the maize starches according to the invention have a peak viscosity of at most 2600, or at most 2500 centipoise (cP).

- 5 In contrast to conventional high-amylose maize starches, which do not have a viscosity forming effect, the maize starches according to the invention may display a peak viscosity comparable to that of wild-type maize plants or at least very similar.

The "peak viscosity" in RVA analysis means, in connection with the present invention, the maximum viscosity measured in centipoise (cP), determined after a measurement
10 time of 2 to 8 minutes.

In a further embodiment, the maize starches according to the invention may have a "trough viscosity" of at least 600, at least 750, or at least 1000 centipoise (cP). In this feature, the maize starches according to the invention may differ markedly both from conventional high-amylose maize starches, which owing to the lack of viscosity
15 development have no, or at any rate only a very low trough viscosity of less than 150 centipoise (cP), as well as from maize starches of wild-type maize plants, which similarly have a markedly lower trough viscosity of less than 1000 centipoise (cP).

The "trough viscosity" in RVA analysis means, in connection with the present invention, the minimum viscosity measured in centipoise (cP), which occurs after
20 reaching the peak viscosity and is determined after a measuring time of 6 to 12 minutes, preferably after 7-12 minutes.

In further embodiments, the maize starches according to the invention have, in RVA analysis, a final viscosity of more than 1000 centipoise (cP), more than 1150 and especially preferably more than 1350 centipoise (cP).

- 25 In further embodiments, the maize starches according to the invention have a final viscosity between 1100 and 2000 centipoise (cP), between 1300 and 1800 centipoise (cP), or between 1450 and 1700 centipoise (cP).

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In further embodiments the maize starches according to the invention have a final viscosity of at least 1400, or of at least 1500 centipoise (cP).

In further embodiments the maize starches according to the invention have a final viscosity of at most 2700, or at most 2500 centipoise (cP).

- 5 In further embodiments the maize starches according to the invention have a final viscosity between 1400 and 2700 centipoise (cP), or between 1500 and 2500 centipoise (cP).

The "final viscosity" means, in connection with the present invention, the viscosity value in centipoise that occurs at the end of the RVA analysis. According to
10 AACC Method 61-02, the end of RVA analysis is reached after a measuring time of 12.5 minutes.

In further embodiments, the maize starches according to the invention have a "breakdown" in RVA analysis between 30 and 500 centipoise (cP), between 40 and 250 centipoise (cP), between 50 and 150 centipoise (cP), or preferably between 50
15 and 200. In this respect the maize starches according to these embodiments differ markedly from wild-type maize starches.

"Breakdown" means, in connection with the present invention, the difference of peak viscosity minus trough viscosity.

In further embodiments of the present invention, the maize starches according to the
20 invention have, in RVA analysis, a setback of less than 350 centipoise (cP), less than 300, less than 200, less than 150, or less than 100 centipoise (cP).

In further embodiments, the maize starches according to the invention have a setback between -100 and 350 centipoise (cP), between 0 and 250 centipoise (cP), between 20 and 175 centipoise (cP), or between 50 and 350.

- 25 The term "setback" denotes, in connection with the present invention, the difference of final viscosity minus trough viscosity.

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The (maize) starches according to the invention in some embodiments thus not only have the advantage that their aqueous suspensions have stable viscosity over a wide temperature range, but also that the undesirable rethickening of the starch suspensions only occurs to a reduced extent, if at all, compared with wild-type
5 starches.

Further embodiments relate to a maize starch which has an apparent amylose content between 35 wt.% and 90 wt.%, between 38 wt.% and 85 wt.%, between 40 wt.% and 80 wt.%, or between 42 wt.% and 75 wt.%, and which displays certain viscosity properties in a Brabender analysis.

10 A Brabender analysis in connection with the present invention is an analysis of the viscosity properties of the starches (according to the invention) using a Brabender visco-amylograph. Apart from RVA analysis, Brabender analysis is the most common method of determining the viscosity properties of starch suspensions. They differ for example with regard to sample quantity, sample volume, stirring speed and
15 analysis time.

In connection with the present invention, Brabender analysis of the starches according to the invention is preferably carried out according to the method described hereinbelow (Brabender analysis).

In further embodiments the maize starches according to the invention have, in
20 Brabender analysis, a peak viscosity between 40 and 200 Brabender units (BUs), between 50 and 150 Brabender units (BUs), or between 60 and 120 Brabender units (BUs).

In further embodiments the maize starches according to the invention have, in Brabender analysis, a peak viscosity of at least 35, or at least 60 Brabender units
25 (BUs).

In further embodiments the maize starches according to the invention have, in Brabender analysis, a peak viscosity of at most 200, or at most 150 BUs.

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The “peak viscosity” in Brabender analysis means, in connection with the present invention, the maximum viscosity measured in Brabender units (BUs), determined after a measurement time of 10 to 80 minutes.

In further embodiments the maize starches according to the invention have, in
5 Brabender analysis, a final viscosity between 40 and 180 Brabender units (BUs), between 50 and 150 Brabender units (BUs), or between 55 and 100 Brabender units (BUs).

In further embodiments the maize starches according to the invention have, in
Brabender analysis, a final viscosity of at least 30, or at least 55 Brabender units
10 (BUs).

In further embodiments the maize starches according to the invention have, in Brabender analysis, a peak viscosity of at most 180, or at most 130 BUs.

The “final viscosity” in Brabender analysis means, in connection with the present invention, the maximum viscosity measured in Brabender units (BUs), determined
15 after a measurement time of 120 minutes.

In further embodiments the maize starch according to the invention has, in Brabender analysis, a measurable pasting temperature of at least 80°C, at least 84°C, or at least 87°C.

In further embodiments, the maize starch according to the invention has, in
20 Brabender analysis, a pasting temperature between 85°C to 97°C, between 86°C and 95°C, or between 87°C and 94°C.

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In contrast to conventional native high-amylose starches (for example from ae maize plants, Hylon VII®), which display little if any gelatinization in Brabender analysis under normal pressure, the starches according to the invention have the advantage that despite the increased amylose content they gelatinize under standard conditions.

- 5 As a result the processability of the starch according to the invention may be greatly improved compared with other high-amylose starches. Therefore gelatinization of the starches according to the invention does not require the same increase in temperature or pressure as required for conventional native high-amylose starches; this may lead to energy savings and therefore cost savings.
- 10 The term "pasting temperature in Brabender analysis" means, in connection with the present invention, that the measured value for the start of viscosity development according to RVA analyses is the temperature which can be determined by means of the standard software from Brabender GmbH & Co. KG under standard settings, preferably by means of the program Micro Visco Amylograph, WinVis-2.4.7,
- 15 VisCorr-2.1.2, Univ.Eval.-1.1.2, which is made available by Brabender GmbH & Co. KG.

- A further possible advantage of the starches according to the invention may be that during processing in which hot rolls are used they can be applied to the rolls as a suspension. Other maize starches with increased amylose content would only
- 20 undergo limited gelatinization, if at all, during this processing, and accordingly cannot be applied as paste or film to said rolls. The starches according to the invention may be particularly suitable for all applications in which the thickening capacity, the gelling properties or the binding properties of added substances are important. Therefore the starch according to the invention may be especially suitable for the production of
- 25 foodstuffs such as bakery products, instant food, puddings, soups, candy, chocolate, ice cream, panada for fish or meat, frozen desserts or extruded snacks.

- Furthermore, the starch according to the invention may be suitable for the production of adhesives, applications in textile processing, as an addition for building materials, for applications in the area of animal feeding, as an additive for cosmetics and in
- 30 paper processing.

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In particular, use of the starch according to the invention for the production of cold-water-soluble pregelatinized starches may also be conceivable. Pregelatinized starches are physically modified starches, which are mainly produced by wet-thermal® treatment. In contrast to native starch, with cold water they form dispersions or pastes
5 or gels, depending on the concentration of pregelatinized starch used and on the type of starch used in the production of the pregelatinized starch. Based on these properties, pregelatinized starches have a number of possible applications in the food industry, as well as in many technical areas. The use of pregelatinized starch, which is also known as cold-swelling starch, instead of native starch, has the advantage in
10 various cases that production processes can be simplified and shortened.

For the production, for example, of instant desserts and puddings, pregelatinized starches are required, which after being stirred into a cold liquid, such as water or milk, very quickly form firm gels, for example in the case of a suet pudding. These requirements are not met by the commercial pregelatinized starches from wheat,
15 potato or maize starch. To achieve the abovementioned properties, for the existing commercially available pregelatinized starches it is necessary to include additives in the pregelatinized starch, such as gelatin, alginate, carrageenan (carrageen) and/or inorganic salts. This use of "additives" may not be necessary to the same extent e.g. after production of pregelatinized starches with starches according to the
20 invention, isolated from plant cells according to the invention.

Compared with gels produced using conventional high-amylose maize starches, the gels according to the invention may have an advantage in that their production may require less energy input and no expensive equipment has to be used in order to generate high temperatures/pressures, as the starches according to the invention
25 already undergo gelatinization in water under normal conditions.

In further embodiments of the present invention the maize starch according to the invention has, after pasting in water, a light transmittance of at least 5, at least 7, or at least 9. In further embodiments the maize starch according to the invention has a light transmittance of less than 25, or less than 20.

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In connection with the present invention, the light transmittance is preferably determined by means of the "Determination of light transmittance" method described below.

5 In some embodiments, the maize starches according to the invention, after suspension and heating, therefore have the advantage that the resulting pastes/gels have an increased light transmittance, i.e. transparency, than pastes/gels prepared by means of high-amylose maize starch from conventional ae maize mutants.

10 In some embodiments, a further advantage of the maize starch according to the invention resides in its increased freeze-thaw stability compared with starch from ae maize mutants.

15 In further embodiments of the present invention the maize starches according to the invention have, after one freeze-thaw cycle, a freeze-thaw stability of less than 70% water loss, or less than 65% water loss. In further embodiments of the present invention the maize starches according to the invention have, after one freeze-thaw cycle, a freeze-thaw stability of more than 40% water loss, or more than 50% water loss.

20 In further embodiments of the present invention the maize starches according to the invention have, after one freeze-thaw cycle, a freeze-thaw stability which is elevated compared with maize starch from ae maize mutants. Increasing the freeze-thaw stability preferably means in this connection a reduction in water loss by at least 5%, preferably by at least 10%.

In connection with the present invention the freeze-thaw stability of the starches according to the invention is preferably determined by the "Determination of freeze-thaw stability" method described below.

25 The swelling power is an important functional property, e.g. in the processing of starches in the food industry.

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It is necessary to distinguish between the swelling power of starch in cold water (e.g. room temperature) and the swelling power in warm or hot water.

Native starches have negligible, if any, swelling power in cold water, whereas selected physically modified (pregelatinized, dried) starches can already swell in cold
5 water.

In connection with the present invention, the term "swelling power" relates to the behavior of starch, preferably native starch, in hot aqueous suspensions, preferably at a temperature of 90°C. In connection with the present invention, the swelling power is preferably determined with a 3% (w/v) starch suspension at a temperature of
10 90°C by the method of Leach et al. (Cereal Chemistry 36, (1959), 534-544). The swelling power is determined as the quotient of the weight of the gel divided by the difference of the initial weight of the starch sample and the soluble constituents:

Swelling power (g/g) = weight of the gel / (initial weight of starch – soluble constituents)

15 Commercially available high-amylose maize starch, such as Hylon VII®, has a far lower swelling power, compared with wild-type maize starch. For Hylon VII® starch with an apparent amylose content of approx. 70%, for example, a swelling power of approx. 6 g/g was determined according to the method of Leach et al. (Cereal Chemistry 36, (1959), 534-544), whereas wild-type maize starch exhibits a
20 swelling power of approx. 22 g/g (Shi et al., Journal of Cereal Science 27, (1998), 289-299).

In some embodiments, the maize starch according to the invention has, compared with conventional high-amylose maize starches, the advantage of a considerably improved swelling power. In further embodiments, the maize starch according to the
25 invention has a swelling power of at least 7 g/g, at least 10 g/g, or at least 12 g/g.

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In further embodiments the maize starch according to the invention has a swelling power between 7 g/g and 20 g/g, between 10 g/g and 18 g/g, between 11 g/g and 15 g/g, or between 11 and 17 g/g.

Owing to the increased swelling power relative to conventional high-amylose maize starches, the maize starches according to some embodiments of the invention may be better suited for certain applications. If, for example, starch is used as a thickening agent, the increased swelling power of the starch means that much less starch can be used to achieve the same thickening effect. This means that for example the calorie content of starch-thickened foodstuffs may be reduced.

10 In further embodiments, the maize starches according to the invention have an increased solubility in hot water, relative to native high-amylose maize starches. In some embodiments, the solubility in hot water of the maize starches according to the invention is at least 8%, at least 9%, or at least 11%.

15 In further embodiments the maize starches according to the invention have a solubility in hot water between 8% and 25%, between 9% and 20%, or between 10% and 16%.

In connection with the present invention, the solubility in hot water is preferably determined with a 3% (w/v) starch suspension at 90°C by the method of Leach et al. (Cereal Chemistry 36, (1959), 534-544), which is also described below ("General methods"). The solubility in hot water is found from the following formula:

Solubility in hot water (%) = weight of dried supernatant / initial weight of starch x 100

In further embodiments, the maize starches according to the invention have a phosphate content in position C6 between 10 and 40 nmol C6P/mg starch (dry weight), between 12 and 35 nmol C6P/mg starch (dry weight), or between 15 and 30 nmol C6P/mg starch (dry weight).

30725-968PPH

The term "phosphate content in position C6" means, in connection with the present invention, the content of phosphate groups that are bound to carbon atom position "6" of the glucose monomers of the starch. Basically, in starch in vivo, positions C3 and C6 of the glucose units can be phosphorylated. In connection with the present invention, the phosphate content in position C6 (= C-6-P content) is preferably determined through glucose-6-phosphate determination by the optical-enzymatic test described later (modified according to Nielsen et al., 1994, Plant Physiol. 105, 111-117).

In further embodiments the (native) maize starch according to the invention has modified digestive properties compared with starches from the corresponding maize wild-type plants.

"Modified digestive properties" compared with starches from the corresponding maize wild-type plants is to be understood in connection with the present invention as meaning in particular an increased content of resistant starch (RS content) and/or a reduced fraction of rapidly digestible starch RDS.

In some embodiments the RS content of the native maize starch according to the invention is comparable to the RS content of corresponding starches from maize plants which have an amylose extender mutation and do not express a heterologous GWD gene.

In further embodiments the native maize starch according to the invention has a content of rapidly digestible starch RDS between 1 wt.% to 20 wt.%, between 2 wt.% to 18 wt.%, or between 3 wt.% to 16 wt.%.

In connection with the present invention the RS and RDS contents are determined and calculated via the method of Englyst et al. (Europ. J. of Clinical Nutrition 46 (Suppl. 2), (1992), S 33-50, see in particular the following sections from Englyst et al., page S35-S36: "Reagents, Apparatus, Spectrophotometer"; page S36-S37, paragraph "Measurement of free glucose (FG)"; page S38, paragraph "Measurement of RDS and SDS"; S39 Measurement of RS₃).

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Since the RDS content of maize starches isolated from maize plants (cells) which express a heterologous GWD gene and have no amylose extender mutation is above that of the corresponding maize wild-type plants, it is surprising to a person skilled in the art that the RDS content of the maize starches in some embodiments according to the invention is comparable to the RDS content of maize starches isolated from maize/wheat plants (cells) having an amylose extender mutation but not expressing a heterologous GWD gene.

A reduced RDS content may also be displayed by processing products (for example food products) of the maize starch according to the invention even when the starch in these products is no longer in the native form.

The reduced RDS content of the maize/wheat starches according to the invention and of the maize flours according to the invention may be of appreciable nutritional advantage. This is because the constant consumption of food products having a high glycemic load, such as, for example, food products containing rapidly digestible starches, and the attendant insulin response are suspected of being a risk factor in the etiology of diseases such as high blood pressure, adipositas, heart diseases and type II diabetes.

The present invention in some embodiments also relates to the maize starch with one or more of the properties described above, produced by the plant cells according to the invention or the plants according to the invention. That is, this description discloses any combination of the following properties of starches: apparent amylose content, phosphate content in position C6, pasting temperature, peak viscosity, trough viscosity, final viscosity, breakdown, setback, swelling power, solubility in hot water, gel strength, RS content, RDS content, freeze-thaw stability, light transmittance. All combinations of two, three, four, five, six, seven, eight, nine and all properties are to be regarded as disclosed.

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A person skilled in the art knows that the properties of starch can be altered by e.g. thermal, chemical, enzymatic or mechanical derivatization. Derivatized starches are particularly suitable for various applications in the food and/or nonfood area. The starches according to the invention may be more suitable as starting substance for
5 the production of derivatized starches than conventional high-amylose starches, as they have, e.g. through the higher content of starch phosphate in some embodiments, a higher proportion of reactive functional groups and, moreover, are more easily gelatinized.

The present invention therefore also relates to methods of production of a derivatized
10 maize starch, in which native maize starch according to the invention is derivatized subsequently. Furthermore, the present invention also relates to derivatized maize starch containing the maize starch according to the invention.

The term "derivatized starch" means, in connection with the present invention, a maize starch according to the invention, whose properties were altered by means of
15 chemical, enzymatic, thermal or mechanical methods after isolation from the maize plants (cells) according to the invention or their reproductive material.

In a further embodiment of the present invention, the derivatized maize starch according to the invention is a heat-treated and/or acid-treated starch.

In a further embodiment, the derivatized maize starch according to the invention is a
20 starch ether, in particular starch alkyl ether, O-allyl ether, hydroxylalkyl ether, O-carboxymethyl ether, nitrogen-containing starch ether, phosphate-containing starch ether or sulfur-containing starch ether.

In a further embodiment, the derivatized maize starches according to the invention are crosslinked starches.

25 In a further embodiment, the derivatized maize starches according to the invention are starch graft polymers.

30725-968PPH

In a further embodiment, the derivatized maize starches according to the invention are oxidized starches.

In a further embodiment, the derivatized maize starches according to the invention are starch esters, in particular starch esters that are introduced into the starch using
5 organic acids. Especially preferably, they are so-called phosphate, nitrate, sulfate, xanthate, acetate or citrate starches.

The derivatized maize starches according to the invention may be suitable for various uses in the pharmaceutical industry, and in the food and/or nonfood area. Methods for production of derivatized maize starches according to the invention are known by
10 a person skilled in the art and are described in the general literature. A review of the production of derivatized starches is given e.g. in Orthoefer (in Corn, Chemistry and Technology, 1987, eds. Watson and Ramstad, Chapter 16, 479-499).

Derivatized starch, obtainable or obtained by the method according to the invention for the production of a derivatized starch, is also an object of the present invention.

15 In addition, the use of modified starches according to the invention for the production of derivatized starch is an object of the present invention.

The present invention also relates to maize plant (cells) that express a heterologous GWD gene and have an amylose extender mutation.

The present invention also relates to maize plants that contain the maize plant cells
20 according to the invention.

In a preferred embodiment the maize plant (cells) according to the invention synthesize the maize starch according to the invention.

In a further preferred embodiment the maize plants (cells) according to the invention are transgenic maize plants (cells), which contain a transgene that leads to a
25 detectable expression of a heterologous GWD gene.

30725-968PPH

In connection with the present invention, a GWD protein means a protein with the enzymatic activity of an alpha-glucan, water dikinase (GWD, E.C.: 2.7.9.4) (Ritte et al., 2002, PNAS 99, 7166-7171), which was often also designated as R1 protein, especially in the earlier scientific literature (e.g. WO9711188, WO9827212, 5 Lorberth et al., Nature Biotechnology 16 (1998), 473-477). In the GWD-catalyzed reaction, the educts of alpha-1,4-glucan or starch, adenosine triphosphate (ATP) and water are converted to the products glucan-phosphate (starch phosphate), monophosphate and adenosine monophosphate (Kötting et al., 2005, Plant Physiol. 137, 2424-252, Ritte et al., 2002, PNAS 99, 7166-7171). In the process, a 10 beta-phosphate residue is transferred from ATP to starch or glucan.

GWD can utilize nonphosphorylated starch as a substrate, i.e. a *de novo* phosphorylation of nonphosphorylated starch can be catalyzed by GWD (Kötting et al., 2005, Plant Physiol. 137, 2424-252, Baunsgaard et al., 2005, Plant Journal 41, 595-605). GWD can insert phosphate groups in position C6 of the 15 glucose monomers of starch.

It is assumed that during catalysis of the phosphorylation reaction of a starch by a protein with the activity of a glucan water dikinase, a phosphorylated protein is formed as an intermediate, in which the beta-phosphate residue of the ATP is bound covalently to an amino acid of the protein with the activity of a glucan water dikinase 20 (Ritte et al., 2002, PNAS 99, 7166-7171). The intermediate arises through autophosphorylation of the protein with the activity of a glucan water dikinase, i.e. the protein with the activity of a glucan water dikinase itself catalyzes the reaction that leads to the intermediate (Ritte et al., 2002, PNAS 99, 7166-7171).

In potato plants, the GWD (R1) protein is bound to the starch granules of the storage 25 starch in potato tubers (Lorberth et al., 1998, Nature Biotechnology 16, 473-477).

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Starches isolated from leaves of an *Arabidopsis thaliana* *sex1-3* mutant do not have any detectable amounts of covalently-bound phosphate residues, but they are phosphorylated in vitro by a protein with the activity of a glucan water dikinase. That is, nonphosphorylated starch, e.g. isolated from leaves of an *Arabidopsis thaliana* *sex1-3* mutant, is utilized as substrate in a phosphorylation reaction catalyzed by a protein with the activity of a glucan water dikinase (Ritte et al., *Planta* 216, (2003), 798-801).

Amino acid sequences that encode proteins with the activity of a glucan water dikinase contain a phosphohistidine domain. Phosphohistidine domains are for example described in Tien-Shin Yu et al. (2001, *Plant Cell* 13, 1907-1918). In the autophosphorylation of a protein with the activity of a glucan water dikinase, a histidine residue in the phosphohistidine domain of the amino acid sequence encoding a protein with the activity of a glucan water dikinase is phosphorylated (Mikkelsen et al., 2004, *Biochemical Journal* 377, 525-532). In the protein sequence of a protein with the activity of a glucan water dikinase from *Solanum tuberosum*, shown as SEQ ID NO 2, for example the amino acids 1064 to 1075 constitute the phosphohistidine domain. If the conserved histidine residue (in the protein sequence shown as SEQ ID NO 2, for example, amino acid 1069) of the phosphohistidine domain is replaced with another amino acid, there is no longer any autophosphorylation and therefore also no phosphorylation of glucans by the mutagenized protein (Mikkelsen et al., 2004, *Biochemical Journal* 377, 525-532). Furthermore, a protein with the activity of a glucan water dikinase is characterized in that it has a C-terminal nucleotide binding domain, which comprises amino acids 1121 to 1464 in the amino acid sequence shown as an example as SEQ ID NO 2. A deletion of the nucleotide binding domain leads to inactivation of a protein with the activity of a glucan water dikinase (Mikkelsen and Blennow, 2005, *Biochemical Journal* 385, 355-361).

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At the N-terminus, proteins with the activity of a glucan water dikinase have a carbohydrate binding domain, also called carbohydrate binding module (CBM), comprising amino acids 78 to 362 in the amino acid sequence shown as SEQ ID NO 2. Among other things, carbohydrate binding domains are characterized

5 in that their amino acid sequences have conserved tryptophan residues. If these conserved amino acid residues are exchanged for other amino acids, the carbohydrate binding domains can lose their ability to bind glucans. Thus, for example, exchange of amino acids W139 or W194 in the amino acid sequence shown as SEQ ID No. 2 leads to a loss of function of this carbohydrate binding

10 domain. If the carbohydrate binding domain of a glucan water dikinase is deleted (for example a deletion of amino acids 1 to 362 of the amino acid sequence shown as SEQ ID No. 2, with amino acids 1 to 77 representing a plastid signal peptide), this does not, however, lead to inactivation of the phosphorylating activity of the enzyme (Mikkelsen et al., 2006, Biochemistry 45, 4674-4682).

15 Nucleic acid sequences and their corresponding amino acid sequences, encoding a protein with the activity of a glucan water dikinase have been described from various species, such as potato (WO9711188, GenBank Acc.: AY027522.1, Y09533.1), wheat (WO0077229, US 6,462,256, GenBank Acc.: AAN93923.1, GenBank Acc.: AR236165.1), rice (GenBank Acc.: AAR61445.1, US 6620987,

20 GenBank Acc.: AR400814.1), maize (GenBank Acc.: AAR61444.1, GenBank Acc.: AR400813.1; WO9827212), soybean (GenBank Acc.: AAR61446.1, GenBank Acc.: AR400815.1), *Curcuma longa* (SEQ ID NOs 3 and 4), citrus (GenBank Acc.: AY094062.1), *Arabidopsis* (GenBank Acc.: AF312027.1), the green alga *Ostreococcus tauri* (GenBank Acc.: AY570720.1) and from

25 *Chlamydomonas reinhardtii* (US60/701,693, WO2007/018770). A synthetic GWD nucleotide sequence optimized for expression in maize was described under SEQ ID No.1 in WO2005/002359 (corresponds to US2006/0282917 A1).

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It is preferable, in connection with the present invention, to use nucleic acid molecules that comprise the entire coding region of a GWD. Said nucleic acid sequences and amino acid sequences encoding a protein with the activity of a glucan water dikinase are published *inter alia* by National Center for Biotechnology

5 Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA.

In connection with the present invention, apart from the GWD genes enumerated here, it is also possible to use GWD genes from other organisms or synthetic GWD genes. Preferably the GWD gene is derived from *Solanum tuberosum* or

10 *Curcuma longa* and has sequence identity of at least 50%, in particular at least 60%, preferably at least 70%, especially preferably of at least 80% and quite especially preferably of at least 90% to the coding region of the nucleic acid molecules given as SEQ ID No. 1 (*Solanum tuberosum*) or as SEQ ID No. 3 (*Curcuma longa*). Moreover, the coding region of the nucleotide sequence given as SEQ ID No. 1 is preferred, and
15 the coding region of the nucleotide sequence given as SEQ ID No. 3 is especially preferred.

In a preferred embodiment, in connection with the maize plants (cells) according to the invention, a GWD of the genus *Curcuma* or *Solanum*, preferably of the species *Curcuma longa* or *Solanum tuberosum*, is used.

20 The GWD with the amino acid sequence given as SEQ ID No. 2 is preferred, and the GWD with the amino acid sequence given as SEQ ID No. 4 is especially preferred.

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The term "identity" means, in connection with the present invention, the number of coinciding amino acids/nucleotides (identity) with other proteins/nucleic acids, expressed as a percentage. The identity of a protein, for example with the activity of a GWD, is preferably determined by comparison with the amino acid sequence given as SEQ ID NO 2 or SEQ ID No. 4 or the identity of a nucleic acid molecule encoding a protein with the activity of a GWD is determined by comparison with the coding nucleic acid sequence given as SEQ ID No. 1 or SEQ ID No. 3 with other proteins/nucleic acids using computer programs. If sequences that are being compared with one another have different lengths, the identity is to be determined so that the number of amino acids/nucleotides which the shorter sequence has in common with the longer sequence, determines the percentage identity. Preferably the identity is determined using the well-known, publicly available computer program ClustalW (Thompson et al., Nucleic Acids Research 22 (1994), 4673-4680). ClustalW is made publicly available by Julie Thompson (Thompson@EMBL-Heidelberg.DE) and Toby Gibson (Gibson@EMBL-Heidelberg.DE), European Molecular Biology Laboratory, Meyerhofstrasse 1, D 69117 Heidelberg, Germany. ClustalW is also available from various institutes, including IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P.163, 67404 Illkirch Cedex, France) and EBI (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK).

It is preferable to use the ClustalW computer program of Version 1.8, for determining the identity between proteins described within the scope of the present invention and other proteins. The following parameters are to be set: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3, GAOPEN=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET, ENDGAPS(OFF), NOPGAP, NOHGAP.

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It is preferable to use the ClustalW computer program of Version 1.8, for determining the identity between e.g. the nucleotide sequence of the nucleic acid molecules described within the scope of the present invention and the nucleotide sequence of other nucleic acid molecules. The following parameters are to be set: KTUPLE=2,
 5 TOPDIAGS=4, PAIRGAP=5, DNAMATRIX:IUB, GAOPEN=10, GAPEXT=5, MAXDIV=40, TRANSITIONS: unweighted.

A "heterologous" GWD gene or protein means, in connection with the present invention, a GWD that does not occur naturally in the maize or wheat plant (cell), but whose coding DNA sequence is inserted in the cell, for example by methods of
 10 genetic engineering, such as transformation of the cell. Then the coding DNA sequence of the heterologous GWD can for example be derived from a different maize variety than the transformed maize plant cell. It is preferably not under the control of its own promoter, but under the control of a promoter that is heterologous with respect to the particular GWD gene. Preferably the heterologous GWD is
 15 derived from a different plant species than the transformed maize plant cell/maize plant according to the invention or the GWD used is under the control of a heterologous promoter, i.e. of a promoter that does not correspond to the natural GWD promoter of the particular GWD. Especially preferably, the coding DNA sequence of the heterologous GWD is derived from a different plant genus than the
 20 transformed maize plant cell/maize plant.

The term "plant genus" means, in connection with the present invention, a hierarchic stage of the system of biological classification. A genus contains one or more species. An example of a genus is *Triticum* L. (wheat). All species within a genus always have a two-part (binomial) name, which in addition to the genus designation
 25 also includes a specific epithet. *Triticum aestivum* L. (bread wheat) is accordingly a species of the genus *Triticum*.

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The term "maize plants (cells) that have an amylose extender mutation" means, in connection with the present invention, maize plants (cells) that have a mutation of the gene of the starch branching enzyme IIb from maize (abbreviated to "BE IIb" or "SBE IIb"), also called amylose extender gene, and this mutation leads to a decrease
 5 in SBE IIb enzyme activity in the endosperm of these maize plants compared to the BE IIb activity in the endosperm of wild-type maize plants.

Preferably this mutation of BE IIb in maize plants (cells) has the effect that no SBE IIb activity is detectable any longer (e.g. Fisher et al., Plant Physiol. 110, (1996), 611-619, in particular Fig. 4; Hedman and Boyer, Biochemical Genetics 21 (11/12),
 10 (1983), 1217-1222, in particular Table 1).

BE IIb protein from maize means, in connection with the present invention, a branching enzyme of the isoform IIb that is encoded by the so-called amylose extender gene (Kim et al., Plant Molecular Biology 38, (1998), 945-956). The branching enzyme (BE) of the isoform IIb (α -1,4-glucan: α -1,4-glucan
 15 6-glycosyltransferase; E.C. 2.4.1.18) catalyzes a transglycosylation reaction, in which α -1,4-linkages of an α -1,4-glucan donor are hydrolyzed and the α -1,4-glucan chains thus released are transferred to an α -1,4-glucan acceptor chain and thus converted to α -1,6-linkages.

In its biochemical properties, the BEIIb protein from maize differs markedly from the
 20 BEI protein from maize, presented by Fisher et al. (Plant Physiol. 110, (1996), 611-619) in Table 1, page 612. For example, the BEI protein amylose branches more quickly than the BEIIb protein, whereas the BEIIb protein amylopectin branches at a higher rate than the BEI protein does (Guan and Preiss, Plant Physiol. 102, (1993), 1269-1273). The amino acid sequence of the BEIIb protein differs from the
 25 BEIIa protein according to Gao et al. (Plant Physiol. 114, (1997), 69-78) mainly by a 49 amino acid long N-terminal extension of the BEIIa protein. The molecular weight of the BEIIa protein determined by SDS-PAGE is 89 kD, and that of the BEIIb protein is somewhat less, namely 85 kDa (Fisher et al., Plant Physiol. 110, (1996), 611-619).

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The amino acid sequence of the BEIIb protein from maize has identity of at least 85%, preferably of at least 90%, of at least 95% and especially preferably of at least 98% with the amino acid sequence shown as SEQ ID NO 6.

5 An "amylose extender" gene (also "BEIIb" gene) from maize means, in connection with the present invention, a gene that encodes a BEIIb protein. The nucleic acid sequence of the amylose extender gene from maize has identity of at least 85%, preferably of at least 90%, of at least 95% and especially preferably of at least 98% with the nucleic acid sequence shown as SEQ ID No. 5.

10 In a further embodiment of the present invention, the "amylose extender mutation" of the maize plant (cell) according to the invention is a dominant mutation of the amylose extender 1 locus, which leads to the synthesis of a maize starch that has an increased apparent amylose content compared with wild-type maize plants (cells), which is between 35 and 90 wt.%, in particular between 38-85 wt.%, preferably 40-80 wt.% and especially preferably between 42-75 wt.%.

15 Preferably, the dominant mutation is the mu-induced allele Ae1-5180 of the amylose extender 1 locus (Stinard et al., Plant Cell 5, (1993), 1555-1566).

20 In an especially preferred embodiment of the present invention, the "amylose extender mutation" of the maize plant (cell) according to the invention is a maize plant (cell) that has a homozygously recessive "amylose extender" genotype and synthesizes a maize starch that has an apparent amylose content between 38-85 wt.%, preferably between 40-80 wt.% and especially preferably between 42-75 wt.%. The amylose extender 1 (ae1) locus includes the structural gene that encodes the SBE IIb protein (Hedman and Boyer, Biochemical Genetics 20 (5/6), (1982), 483-492).

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Amylose extender (ae) maize mutants were described for example in Vineyard and Bear (Maize Genet Coop Newsletter 26: 5 (1952), who describe the reference allele ae1-Ref, and in Moore and Creech (Genetics 70, (1972), 611-619), Garwood et al. (Cereal Chemistry 53(3), (1976), 355-364) and Hedman and Boyer
5 (Biochemical Genetics 21 (11/12), (1983), 1217-1222).

In a further embodiment of the present invention, the maize plant (cell) according to the invention, which synthesizes the maize starch according to the invention, is genetically modified, wherein the genetic modification with reference to maize leads to a detectable expression of the heterologous GWD and to a decrease in activity of
10 the BEIIb protein compared with corresponding wild-type maize plants (cells) that are not genetically modified.

The genetic modification can be any genetic modification that leads, with reference to maize, to a detectable expression of the heterologous GWD and to a decrease in activity of the BEIIb protein compared with corresponding wild-type maize plants
15 (cells) that are not genetically modified.

The term "detectable expression of a heterologous GWD" means, within the scope of the present invention, that the expression of a heterologous GWD can be determined by measurement of the quantity of transcripts of the heterologous GWD, e.g. by Northern Blotting or RT-PCR, and that the expression of the heterologous GWD
20 leads to an increase in the phosphate content in position C6 of the starch compared with starch from wild-type plants (cells) that do not express heterologous GWD.

An increase in phosphate content in position C6 of the starch means, in connection with the present invention, an increase in phosphate content in position C6 of the starch by at least 100%, preferably by at least 1000% and especially preferably by at
25 least 1500%, even more especially preferably by at least 7000% compared with the phosphate content in position C6 of the starch from corresponding wild-type plants (cells).

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In a further embodiment of the present invention, the detectable expression of a heterologous GWD results in an increase in the enzymatic GWD activity of the respective plant (cell) according to the invention compared to the GWD activity of a corresponding wild-type plant (cell) and/or in an increase in the amount of proteins
 5 with the activity of a GWD in the maize plants (cells) according to the invention.

The amount of GWD protein can be determined for example by Western Blotting, and the enzymatic activity of a GWD can be determined for example as described in Ritte et al. (Ritte et al., *Planta* 216, (2003), 798-801).

The term "decrease in activity of the BEIIb protein" means, within the scope of the
 10 present invention, with reference to maize a decrease in expression of endogenous genes that encode the BEIIb protein and/or a decrease in the amount of BEIIb protein in the maize plants (cells) according to the invention and/or preferably a decrease in enzymatic activity of BEIIb protein in the maize plants (cells) according to the invention.

15 The increase/decrease in expression can be determined for example by measuring the quantity of transcripts that encode proteins with the activity of a GWD or of a BEIIb protein or of a BEIIa protein. Determination can be carried out e.g. by Northern Blotting or RT-PCR.

Determination of the BEIIb activity of maize plants takes place, in connection with the
 20 present invention, preferably after fractionation of the various isoforms of the branching enzyme from maize, as in Fisher et al., *Plant Physiology* 110, (1996), 611-619), in particular as in the sections described there "Reagents Used in Assays", "SBE Purification", "SBE Activity Assay" and "SBE Activity Analysis of Selected Alleles".

25 The decrease in enzymatic activity of BEIIa and/or BEIIb protein is detected, in connection with the present invention, especially preferably with the aid of activity gels, as described below under "General Methods".

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A decrease in enzymatic activity of BEIIb proteins means in connection with the present invention, with reference to maize, preferably a decrease in enzymatic activity that leads to an apparent amylose content between 35 and 90 wt.%, in particular between 38-85 wt.%, preferably of 40-80 wt.% and especially preferably
5 between 42-75 wt.%.

The term "wild-type maize plant cell" means, in connection with the present invention, that we are dealing with maize plant cells that served as starting material for the production of the maize plant cells according to the invention, which synthesize the starch according to the invention. The term "wild-type maize plant cell" does not
10 include, in connection with the present invention, any maize plant cells from maize mutants of the ae (amylose extender), wx (waxy), du (dull), sh2 (shrunk 2), brittle-1 or of the brittle-2 genotype or of double or multiple mutants of these genotypes. The term "wild-type maize plant" means, in connection with the present invention, that we are dealing with maize plants that served as starting material for the production of the
15 maize plants according to the invention, which synthesize the starch according to the invention. The term "wild-type maize plant" does not include, in connection with the present invention, any maize mutants of the ae (amylose extender), wx (waxy), du (dull), sh2 (shrunk 2), brittle-1 or of the brittle-2 genotype or of double or multiple mutants of these genotypes.

20 The term "wild-type maize plant" preferably relates to the maize inbreeding line A188, which is publicly available, for example from the Maize Genetics Cooperation Stock Center at the University of Illinois, Urbana/Champaign, S-123 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801-4730, USA.

30725-968PPH

The term "corresponding" means, in connection with the present invention, that when comparing several objects, the objects in question, that are being compared with one another, are kept under identical conditions. In connection with the present invention, the term "corresponding" in the context of wild-type maize plant cell or wild-type
5 maize plant, means that the plant cells or plants that are being compared with one another were bred under identical cultivation conditions and that they have an identical (cultivation) age.

In a further embodiment of the present invention, the "amylose extender mutation" and/or the increase in the activity or expression of a GWD of the plants (cells)
10 according to the invention is brought about by mutagenesis of one or more genes. The type of mutation is irrelevant, provided that, with reference to the maize plants according to the invention, it leads to a decrease in BEIIb activity and/or to an increase in the expression or activity of a GWD.

The term "mutagenesis" means, in connection with the present invention, introduced
15 mutations of any kind, such as deletions, point mutations (nucleotide substitutions), insertions, inversions, gene conversions or chromosome translocations.

A mutation that leads to a decrease in BEIIa and/or BEIIb activity and/or to an increase in the expression or activity of a GWD can arise spontaneously in a plant, and the corresponding plants can be selected and propagated using the methods
20 described below.

A mutation that leads to a decrease in BEIIa and/or BEIIb activity and/or to an increase in the expression or activity of a GWD can also be produced through the use of chemical agents or high-energy radiation (e.g. X-rays, neutron, gamma, or UV radiation).

25 Agents that can be used for the production of chemically induced mutations, and the mutations resulting from the action of the corresponding mutagens, are described for example by Ehrenberg and Husain (1981, Mutation Research 86, 1-113) and Müller (1972, Biologisches Zentralblatt 91 (1), 31-48).

30725-968PPH

- The production of rice mutants using gamma rays, ethyl methane sulfonate (EMS), N-methyl-N-nitrosourea or sodium azide (NaN_3) is described for example by Jauhar and Siddiq (1999, Indian Journal of Genetics, 59 (1), 23-28), Rao (1977, Cytologica 42, 443-450), Gupta and Sharma (1990, Oryza 27, 217-219) and
- 5 Satoh and Omura (1981, Japanese Journal of Breeding 31 (3), 316-326). The production of wheat mutants using NaN_3 or maleic acid hydrazide is described by Arora et al. (1992, Annals of Biology 8 (1), 65-69). A review of the production of wheat mutants using various types of high-energy radiation and chemical agents is presented by Scarascia-Mugnozza et al. (1993, Mutation Breeding Review 10, 1-28).
- 10 Svec et al. (1998, Cereal Research Communications 26 (4), 391-396) describe the use of N-ethyl-N-nitrosourea for the production of mutants in triticale. The use of MMS (methyl methanesulfonic acid) and gamma radiation for the production of millet mutants is described by Shashidhara et al. (1990, Journal of Maharashtra Agricultural Universities 15 (1), 20-23).
- 15 In connection with the present invention, plants (cells) according to the invention can also be produced using the so-called insertion mutagenesis (review article: Thorneycroft et al., 2001, Journal of Experimental Botany 52 (361), 1593-1601). Insertion mutagenesis, in connection with the present invention, means in particular the insertion of transposons or so-called transfer DNA (T-DNA) in a gene or in the
- 20 vicinity of a gene encoding a protein with the activity of a GWD or of a BEIIb or of a BEIIa, whereby the activity of a protein with the activity of a GWD is increased in the cell in question or the activity of the BEIIb protein or of a BEIIa protein is decreased.
- The transposons can either be those that occur naturally in the cell (endogenous transposons), or that do not occur naturally in said cell, but were introduced into the
- 25 cell by methods of genetic engineering, such as transformation of the cell (heterologous transposons). Alteration of the expression of genes by transposons is known by a person skilled in the art. A review of the use of endogenous and heterologous transposons as tools in plant biotechnology is presented in Ramachandran and Sundaresan (2001, Plant Physiology and Biochemistry 39,
- 30 234-252).

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T-DNA insertion mutagenesis is based on the fact that certain segments (T-DNA) of Ti-plasmids from *Agrobacterium* can be integrated into the genome of plant cells. The place of integration in the plant chromosome is not fixed, but can take place at any point. If the T-DNA is integrated into a segment or in the vicinity of a segment of the chromosome that represents a gene function, this can lead to increase or decrease in gene expression and hence to a change in the activity of a protein encoded by the gene in question.

The sequences (in particular transposons or T-DNA) inserted into the genome are characterized in that they contain sequences that lead to activation of regulatory sequences of a gene that encodes a protein with the activity of a GWD ("activation tagging"). Preferably sequences inserted into the genome (in particular transposons or T-DNA) are characterized in that they are integrated in the vicinity of endogenous nucleic acid molecules in the genome of the plant cell or of the plant, which encode a protein with the activity of a GWD.

Plants (cells) according to the invention can also for example be produced by the method of so-called "activation tagging" (see e.g. Walden et al., *Plant J.* (1991), 281-288; Walden et al., *Plant Mol. Biol.* 26 (1994), 1521-1528). This method is based on the activation of endogenous promoters by "enhancer" sequences, such as the enhancer of the 35S RNA promoter of the cauliflower mosaic virus or the octopine synthase enhancer.

The term "T-DNA activation tagging" means, in connection with the present invention, a T-DNA fragment that contains "enhancer" sequences and, through integration in the genome of a plant cell, leads to an increase in the expression or activity of a protein with the activity of a GWD.

The term "genome" means, in connection with the present invention, all of the hereditary material present in a plant cell. It is known by a person skilled in the art that in addition to the cell nucleus, other compartments (e.g. plastids, mitochondria) also contain hereditary material.

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The term "transposon activation tagging" means, in connection with the present invention, a transposon that contains "enhancer" sequences and, through integration in the genome of a plant cell, leads to an increase in the expression or activity of a protein with the activity of a GWD.

- 5 It is known by a person skilled in the art that in the case of polyploid plants, such as wheat, in certain circumstances expression of the amylose extender phenotype requires three nonfunctional BEIIa or BEIIa and BEIIb mutations (on the A, B and D sub-genome) to be present homozygously.

10 All of these methods are basically suitable for production of the "amylose extender mutation" and/or for increasing the expression or activity of a GWD and therefore for the production of the plant cells according to the invention or the plants according to the invention.

Mutations in the corresponding genes, in particular in genes that encode BEIIa, BEIIb or a GWD, can be detected by methods that are known by a person skilled in the art.

15 For this it is possible in particular to employ analyses based on hybridizations with probes ("Southern Blot"), amplification by polymerase chain reaction (PCR), sequencing of relevant genomic nucleic acid fragments and searching for individual nucleotide substitutions. A method of identifying mutations on the basis of hybridization patterns is e.g. searching for restriction fragment length differences

20 (restriction fragment length polymorphism, RFLP) (Nam et al., 1989, The Plant Cell 1, 699-705; Leister and Dean, 1993, The Plant Journal 4 (4), 745-750). A PCR-based method is e.g. analysis of amplified fragment length differences (amplified fragment length polymorphism, AFLP) (Castiglioni et al., 1998, Genetics 149, 2039-2056; Meksem et al., 2001, Molecular Genetics and Genomics 265, 207-214; Meyer et al.,

25 1998, Molecular and General Genetics 259, 150-160). Amplified fragments cleaved with restriction endonucleases (cleaved amplified polymorphic sequences, CAPS) can also be employed for the identification of mutations (Konieczny and Ausubel, 1993, The Plant Journal 4, 403-410; Jarvis et al., 1994, Plant Mol. Biol. 24, 685-687;

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Bachem et al., 1996, The Plant Journal 9 (5), 745-753). Methods for the determination of SNPs have been described by, among others: Qi et al. (2001, Nucleic Acids Research 29 (22), e116), Drenkard et al. (2000, Plant Physiology 124, 1483-1492) and Cho et al. (1999, Nature Genetics 23, 203-207). In particular, methods are suitable that make it possible to investigate many plants in a short time for mutations in particular genes. Such a method, called TILLING ("Targeting Induced Local Lesions in Genomes") has been described by McCallum et al. (2000, Plant Physiology 123, 439-442).

All of these methods are basically suitable for the identification of plant cells according to the invention or of plants according to the invention.

In a further embodiment of the present invention, the genetic modification of the maize plant (cell) according to the invention comprises the introduction of at least one foreign nucleic acid molecule into the genome of the maize plant cell or into the genome of the maize plant.

In this context, the term "genetic modification" means, with reference to GWD, the insertion of at least one foreign nucleic acid molecule into the genome of a maize plant (cell), with said insertion of said molecule leading to an increase in the expression or activity of a protein with the activity of a GWD. With reference to the amylose extender mutation in maize, a foreign nucleic acid molecule can be any nucleic acid molecule that causes a decrease in BEIIb activity in the plant cell or plant.

Through insertion of a foreign nucleic acid molecule, the maize plants (cells) according to the invention are altered in their genetic information.

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- The presence or the expression of at least one foreign nucleic acid molecule leads to a phenotypic modification. "Phenotypic modification" preferably means a measurable change of one or more functions of the cells. For example, the genetically modified maize plants (cells) can, with reference to GWD, owing to the presence or on
- 5 expression of inserted foreign nucleic acid molecules, show an increase in the expression or activity of a protein with the activity of a GWD. With reference to the BEIIb protein, the maize plants (cells) according to the invention display, owing to the presence or the expression of inserted foreign nucleic acid molecules, a decrease in BEIIb activity.
- 10 The term "foreign nucleic acid molecule" means, in connection with the present invention, such a molecule that either does not occur naturally in corresponding wild-type plant cells, or that does not occur naturally in the precise spatial arrangement in wild-type plant cells or that is localized at a place in the genome of the wild-type plant cell at which it does not occur naturally. Basically, a foreign
- 15 nucleic acid molecule can be, with reference to the GWD, any nucleic acid molecule that causes an increase in the expression or activity of a protein with the activity of a GWD in the plant cell or plant. With reference to the amylose extender mutation in maize, a foreign nucleic acid molecule can be any nucleic acid molecule that causes a decrease in BEIIb activity in the plant cell or plant.
- 20 Preferably the foreign nucleic acid molecule is a recombinant nucleic acid molecule, consisting of various elements, whose combination or specific spatial arrangement does not occur naturally in plant cells.

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The term "recombinant nucleic acid molecule" means, in connection with the present invention, a nucleic acid molecule that has various nucleic acid molecules that do not occur naturally in a combination as in a recombinant nucleic acid molecule. Thus, the recombinant nucleic acid molecules have, for example in addition to the nucleic acid molecules that encode a protein with the activity of a GWD and/or a BEIIb protein or fragments thereof (e.g. genomic nucleic acid molecules or cDNAs), additional nucleic acid sequences that do not occur naturally in combination with these nucleic acid molecules. The recombinant nucleic acid molecule has, for example, regulatory sequences (e.g. promoters, termination signals, enhancers), preferably regulatory sequences that are heterologous with respect to the nucleic acid molecule that encodes the GWD and/or the BEIIb protein or fragments of this protein.

Heterologous means, in this context, that the regulatory sequence is not the actual endogenous regulatory sequence of the GWD gene and/or BEIIb gene and/or BEIIa gene that is used. Moreover, regulatory sequences are preferred that are active in plant tissue, preferably in the endosperm.

Methods for the production of recombinant nucleic acid molecules are known by a person skilled in the art and include methods of genetic engineering, such as the joining of nucleic acid molecules by ligation, genetic recombination or the new synthesis of nucleic acid molecules (see e.g. Sambrook et al., Molecular Cloning, A Laboratory Manual, 3rd edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. ISBN: 0879695773, Ausubel et al., Short Protocols in Molecular Biology, John Wiley & Sons; 5th edition (2002), ISBN: 0471250929).

The foreign nucleic acid molecule or molecules used for genetic modification can be a composite or several separate nucleic acid constructs, in particular so-called single, double or triple constructs. Thus, the foreign nucleic acid molecule can be for example a so-called "double construct", by which we mean a single vector for plant transformation, which contains the genetic information both for decrease/inhibition of BEIIb activity and for increase in the expression or activity of the GWD.

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In a further embodiment of the invention, instead of inserting a double construct in the genome of the plant cell, several different foreign nucleic acid

molecules/polynucleotides are inserted in succession, where one of these foreign nucleic acid molecules is for example a DNA molecule that causes an increase in the
5 expression or activity of the GWD, and a second foreign nucleic acid molecule for example encodes an antisense-RNA, which mediates a decrease in BEIIb activity.

Basically, in the construction of said second foreign nucleic acid molecules, however, cosuppression, ribozymal or double-stranded RNA constructs or in vivo mutagenesis are suitable, provided they lead to a decrease in BEIIb activity.

- 10 The foreign nucleic acid molecules can be inserted into the genome of the plant cell at the same time ("cotransformation") or successively, i.e. one after another ("supertransformation").

The foreign nucleic acid molecules/polynucleotides can also be introduced into various individual plants of a species. It is then possible first to produce plants for

- 15 which the expression of a heterologous GWD is detectable or for which the BEIIb activity is reduced. In an especially preferred embodiment of the present invention, using subsequent crossing, maize plants are produced for which the expression of a heterologous GWD is detectable and the BEIIb activity is reduced.

Furthermore, for introducing a foreign nucleic acid molecule/polynucleotide or for
20 production of the plant cells according to the invention or the plants according to the invention, instead of a wild-type plant cell or plant it is possible to employ a mutant, which is characterized in that it already has a reduced BEIIb activity or expression of a heterologous GWD. For example, the use of maize amylose extender mutants is basically conceivable, into which a foreign nucleic acid molecule is inserted, which
25 leads to an increase in the expression or activity of the GWD compared with corresponding wild-type plants (cells).

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In a further embodiment of the present invention, the presence and/or the expression of one or more foreign nucleic acid molecules/polynucleotides leads, relative to the maize plants (cells) according to the invention, to a decrease in BEIIb activity.

This can be achieved by various methods that are known to a person skilled in the art. These include for example the expression of a corresponding antisense-RNA, or of a double-stranded RNA construct, the provision of molecules or vectors that mediate a cosuppression effect, the expression of a correspondingly constructed ribozyme, which specifically cleaves transcripts that encode BEIIb, or so-called "in vivo mutagenesis". Moreover, the decrease in BEIIb activity can also be brought about by simultaneous expression of sense and antisense RNA molecules of the BEIIb (amylose extender) gene. A person skilled in the art is familiar with these methods.

Furthermore, it is known that in planta the formation of double-stranded RNA molecules of promoter sequences in trans can lead to methylation and transcriptional inactivation of homologous copies of said promoter (Mette et al., 2000, EMBO J. 19, 5194-5201).

For inhibition of gene expression by antisense or cosuppression technology, it is possible for example to use a DNA molecule that includes the complete sequence coding for BEIIb, as well as DNA molecules that only include portions of the coding sequence, but these portions must be long enough to produce an antisense effect or cosuppression effect in the cells. Sequences with a minimum length of 21-23 bp, preferably with a minimum length of 50 bp, especially preferably with a length of 100-500 bp are generally suitable.

For antisense or cosuppression approaches it is also appropriate to use polynucleotide sequences that have a high degree of identity to the sequences occurring endogenously in the plant cell, which code for BEIIb. The minimum identity should be greater than approx. 90%. The use of sequences with identities of at least 95%, in particular of at least 98% is to be preferred.

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Moreover, to achieve an antisense or a cosuppression effect it is also conceivable to use introns, i.e. noncoding regions of genes that code for BEIIb.

The use of intron sequences for the inhibition of the expression of genes that encode proteins of the starch biosynthesis was described in WO 97/04112, WO 97/04113,
5 WO 98/37213, WO 98/37214.

A person skilled in the art knows how to achieve an antisense and a cosuppression effect. The method of cosuppression inhibition was described for example by Jorgensen (1990, Trends Biotechnol. 8, 340-344), Niebel et al. (1995, Top. Microbiol. Immunol. 197, 91-103), Flavell et al. (1995, Curr. Top. Microbiol. Immunol. 197,
10 43-46), Palauqui and Vaucheret (1995, Plant Mol. Biol. 29, 149-159), Vaucheret et al. (1995, Mol. Gen. Genet. 248, 311-317), de Borne et al. (1994, Mol. Gen. Genet. 243, 613-621).

The expression of ribozymes to reduce the activity of particular enzymes in cells is also known by a person skilled in the art and is described for example in
15 EP-B1 0321201. The expression of ribozymes in plant cells was for example described by Feyter et al. (1996, Mol. Gen. Genet. 250, 329-338).

Furthermore, reduction of BEIIb activity in plant cells can also be achieved by so-called "in vivo mutagenesis", in which transformation of cells is used for inserting a hybrid RNA-DNA oligonucleotide ("chimeroplast") in cells (Kipp et al., Poster Session
20 at the 5th International Congress of Plant Molecular Biology, 21-27 September 1997, Singapore; R. A. Dixon and C. J. Arntzen, Meeting report on Metabolic Engineering in Transgenic Plants, Keystone Symposia, Copper Mountain, CO, USA, 1997, TIBTECH 15, 441-447; WO 95/15972; Kren et al., 1997, Hepatology 25, 1462-1468; Cole-Strauss et al., 1996, Science 273, 1386-1389; Beetham et al., 1999, PNAS 96,
25 8774-8778).

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A portion of the DNA component of the RNA-DNA oligonucleotide is homologous to a polynucleotide sequence of an endogenous BEIIb gene but has, compared to the polynucleotide sequence of an endogenous BEIIb gene, a mutation or contains a heterologous region that is surrounded by the homologous regions. By means of
 5 base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous polynucleotide, followed by homologous recombination, the mutation or heterologous region contained in the DNA component of the RNA-DNA oligonucleotide can be transferred into the genome of a plant cell. This leads to a decrease in activity of BEIIb.

10 Moreover, decrease in BEIIb activity in the plant cells can also be brought about by the simultaneous expression of sense and antisense RNA molecules of the particular target gene that is to be repressed, preferably of the BEIIb (amylose extender) gene.

This can for example be achieved through the use of chimeric constructs, which contain "inverted repeats" of the particular target gene or portions of the target gene.

15 The chimeric constructs then encode sense and antisense RNA molecules of the particular target gene. Sense and antisense RNA are synthesized *in planta* simultaneously as one RNA molecule, wherein sense and antisense RNA are separated from each other by a spacer and can form a double-stranded RNA molecule (RNAi technology).

20 It has been shown that the insertion of inverted-repeat DNA constructs in the genome of plants is a very efficient method for repressing the genes corresponding to the inverted-repeat DNA constructs (Waterhouse et al., 1998, Proc. Natl. Acad. Sci. USA 95, 13959-13964; Wang and Waterhouse, 2000, Plant Mol. Biol. 43, 67-82; Singh et al., 2000, Biochemical Society Transactions 28 (6), 925-927; Liu et al., 2000,
 25 Biochemical Society Transactions 28 (6), 927-929; Smith et al., 2000, Nature 407, 319-320; WO 99/53050). Sense and antisense sequences of the target gene or target genes can also be expressed separately from each other by the same or different promoters (Nap et al., 6th International Congress of Plant Molecular Biology, 18-24 June 2000, Quebec, Poster S7-27, Report Session S7).

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Decrease in BEIIb activity can therefore also be achieved through the production of double-stranded RNA molecules of the BEIIb gene. For this, preferably "inverted repeats" of DNA molecules that have been derived from BEIIb genes are inserted in the genome of plants, the DNA molecules that are to be transcribed being under the
5 control of a promoter, which regulates the expression of said DNA molecules.

Furthermore, it is known that the formation of double-stranded RNA molecules of promoter-DNA molecules in plants in trans can lead to a methylation and a transcriptional inactivation of homologous copies of these promoters, which will be designated as target promoters hereinafter (Mette et al., 2000, EMBO J. 19,
10 5194-5201).

Through inactivation of the target promoter it is thus possible to reduce gene expression of the BEIIb gene (target gene), which is naturally under the control of this target promoter.

That is, the DNA molecules that include the target promoters of the genes that are to
15 be repressed (target genes) are in this case, in contrast to the original function of promoters in plants, used not as regulating elements for the expression of genes or cDNAs, but themselves as transcribable DNA molecules.

For the production of the double-stranded target promoter RNA molecules in planta, which may be present there as RNA hairpin molecules, it is preferable to use
20 constructs that contain "inverted repeats" of the target promoter-DNA molecules, with the target promoter-DNA molecules being under the control of a promoter that regulates the gene expression of said target promoter-DNA molecules. These constructs are then inserted in the genome of plants. Expression of the "inverted repeats" of said target promoter-DNA molecules leads *in planta* to the formation of
25 double-stranded target promoter-RNA molecules (Mette et al., 2000, EMBO J. 19, 5194-5201). In this way the target promoter can be inactivated.

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Decrease in BEIIb activity in the plant cells can thus also be achieved through the production of double-stranded RNA molecules of promoter sequences of the BEIIb gene. For this, preferably "inverted repeats" of promoter-DNA molecules of BEIIb promoters are inserted in the genome of plants, with the target promoter-DNA

5 molecules that are to be transcribed (BEIIb promoter) being under the control of a promoter that regulates the expression of said target promoter-DNA molecules.

Moreover, it is known by a person skilled in the art that it is possible to achieve a decrease in BEIIb activity through the expression of nonfunctional derivatives, in particular transdominant mutants, of the enzymes and/or through the expression of

10 antagonists/inhibitors of the enzymes.

Antagonists/inhibitors of the enzymes can for example be antibodies, antibody fragments or molecules with similar binding properties. For example, a cytoplasmic scFv-antibody was used in order to modulate the activity of the phytochrome A-protein in genetically engineered tobacco plants (Owen, 1992, Bio/Technology 10, 790-794; Review: Franken et al., 1997, Current Opinion in Biotechnology 8, 411-416; Whitelam, 1996, Trends Plant Sci. 1, 268-272).

15

Suitable promoters in connection with the present invention are constitutive promoters, such as the promoter of the 35S RNA of the cauliflower mosaic virus (Odell et al., 1985, Nature, 313, 810-812), the ubiquitin promoter from maize

20 (Christensen et al., Plant Mol. Biol. 18, (1992), 675-689), the ubiquitin promoter from rice (Liu et al., Plant Science 165, (2003), the rice actin promoter (Zhang, et al., Plant Cell 3:1150-1160, 1991), the cassava vein mosaic virus (CVMV) promoter (Verdaguer et al., Plant Mol. Biol. 31: 1129-1139), the maize histon H3C4 promoter (US 6,750,378) or the *Cestrum* YLCV promoter (yellow leaf curling virus;

25 WO 01 73087; Stavolone et al., 2003, Plant Mol. Biol. 53, 703-713).

Especially preferably they are tissue-specific regulatory sequences, which are active in maize or wheat tissue, preferably in the endosperm of maize or wheat plants.

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- Other endosperm-specific promoters in maize or wheat are the promoter of the 10 kD zein gene from maize (Kiriwara et al. (1988) Gene 71: 359-370), of the 15 kD zein gene from maize (Hoffmann et al. (1987) EMBO J. 6: 3213-3221; Schernthaner et al. (1988) EMBO J. 7: 1249-1253; Williamson et al. (1988) Plant Physiol. 88: 1002-1007), of the 27 kD zein gene from maize (Prat et al. (1987) Gene 52: 51-49; Gallardo et al. (1988) Plant Sci. 54: 211-281), of the 19 kD zein gene from maize (Marks et al. (1985) J. Biol. Chem. 260: 16451-16459). The relative transcriptional activities of these promoters in maize are described in Kodrzyck et al., (1989), Plant Cell 1, 105-114).
- 10 Other promoters that are conceivable in conjunction with the present invention are the promoter of the sucrose synthase gene (Yang, N.-S. And Russel, D. (1990) Proc. Natl. Acad Sci 87: 4144-4148), of the waxy gene (Unger et al. (1991) Plant Physiol. 96: 124), of the sh 2 gene (Bhave et al. (1990) Plant Cell 2: 581-588, of the bt 2 gene (Bae et al. (1990) Maydica 35: 317-322). Also the HMG promoter (also
- 15 called wheat glutenin HMWG promoter) from wheat (Colot et al., EMBO J. 6, (1987, 3559-3564; Clarke and Appels, Genome 41, (1998), 865-871), the USP promoter, the phaseolin promoter, promoters of zein genes from maize (Pedersen et al., Cell 29 (1982), 1015-1026; Quatroccio et al., Plant Mol. Biol. 15 (1990), 81-93), the glutelin promoter (Leisy et al., Plant Mol. Biol. 14 (1990), 41-50; Zheng et al., Plant J. 4
- 20 (1993), 357-366; Yoshihara et al., FEBS Lett. 383 (1996), 213-218), the globulin promoter (Nakase et al., 1996, Gene 170(2), 223-226) or the prolamine promoter (Qu and Takaiwa, 2004, Plant Biotechnology Journal 2(2), 113-125).

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Intron sequences can also be present between the promoter and the coding region. Said intron sequences can lead to stability of expression and to increased expression in plants (Callis et al., 1987, *Genes Devel.* 1, 1183-1200; Luehrsen, and Walbot, 1991, *Mol. Gen. Genet.* 225, 81-93; Rethmeier, et al., 1997; *Plant Journal.*

- 5 12(4):895-899; Rose and Beliakoff, 2000, *Plant Physiol.* 122 (2), 535-542; Vasil et al., 1989, *Plant Physiol.* 91, 1575-1579; XU et al., 2003, *Science in China Series C Vol. 46 No. 6*, 561-569). Suitable intron sequences are for example the first intron of the sh1 gene from maize (Maas et al. (1991) *Plant. Mol. Biol.* 16: 199-207, the first intron of the poly-ubiquitin gene 1 from maize, the first intron of the EPSPS
10 gene from rice or one of the first two introns of the PAT1 gene from *Arabidopsis*, also introns of the Adh-1 or Bz-1 gene from maize (Callis et al. (1987) *Genes Dev.* 1: 1183-1200), intron 3 of the maize actin gene (Luehrsen, K.R. and Walbot, V. (1991) *Mol. Gen. Genet.* 225: 81-93) or of the Adh1 intron 6 (Oard et al. (1989) *Plant Cell Rep* 8: 156-160).

- 15 The present invention also relates to reproductive material of plants according to the invention.

The term "reproductive material" includes in connection with the present invention preferably endosperm-containing seeds (grains) of the maize plants according to the invention.

- 20 In a further embodiment, the present invention relates to a method of production of the maize starch according to the invention comprising the step of extraction of the starch from a maize plant according to the invention and/or from a maize plant cell according to the invention.

- 25 In a further embodiment of the present invention, the maize starch according to the invention is extracted from a maize plant according to the invention containing maize plant cells according to the invention, from reproductive material of a maize plant according to the invention and/or from starch-storing parts of a maize plant according to the invention.

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The term "starch-storing parts" means, in connection with the present invention, those parts of a plant in which starch, as opposed to transient leaf starch, is stored as reserve for perennation for quite long periods. Preferred starch-storing plant parts are maize grains, and maize grains containing an endosperm are especially
5 preferred.

Preferably the method according to the invention also includes the step of harvesting the cultivated maize plants according to the invention or the starch-storing plant parts and/or the reproductive material of the maize plants according to the invention prior to extraction of the starch. In a further embodiment, the method according to the
10 invention also includes the step of cultivation of the maize plants prior to harvesting.

Methods of extraction of the starch from plants according to the invention or from starch-storing parts of plants according to the invention are known by a person skilled in the art. For example, methods of extraction of the starch from various starch-storing plants are described, e.g. in Starch: Chemistry and Technology
15 (Publ.: Whistler, BeMiller and Paschall (1994), 2nd edition, Academic Press Inc. London Ltd; ISBN 0-12-746270-8; see e.g. Chapter XII, pages 412-468: Maize and Sorghum Starches: Production; by Watson; Chapter XV, pages 491 to 506: Wheat Starch: Production, Modification and Uses; by Knight and Oson) or in Eckhoff et al., Cereal Chem. 73 (1996), 54-57). Extraction of maize starch on an
20 industrial scale is as a rule achieved by means of so-called "wet milling". Devices that are usually employed for the extraction of starch from plant material are separators, decanters, hydrocyclones, spray dryers and fluidized-bed dryers.

In a further embodiment, the present invention relates to maize meal (= maize flour) containing the maize starch according to the invention.

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Starch-storing parts of plants can be processed into meals (flours). For the production of maize meals, the endosperm-containing maize grains are ground and sieved. Starch is a principal constituent of the endosperm. The maize starch according to the invention is, apart from proteins and lipids, the most important
5 constituent of the maize meal (flour) according to the invention (approx. 65 to 75 wt.% of the dry weight of the meal(flour)). The properties of the maize meals (flours) according to the invention are therefore greatly influenced by the maize starch according to the invention contained in the maize meal (flour).

The term "maize meal (= maize flour)" means, in connection with the present
10 invention, a powder obtained by grinding maize grains of maize plants according to the invention, wherein the maize grains consist of maize plant cells according to the invention. Optionally, the maize grains are dried before grinding and are comminuted and/or sieved after grinding.

A further object of the present invention is a method of production of the maize meal
15 (= maize flour) according to the invention comprising the step of grinding at least one maize plant according to the invention. In a further preferred embodiment of the method according to the invention, for the production of the maize meal (= maize flour) according to the invention, maize grains that consist of maize plant cells according to the invention are ground.

20 Preferably, the method according to the invention for the production of maize meals (flours) according to the invention also includes the step of harvesting the maize plants according to the invention or the maize grains according to the invention of these maize plants before grinding, preferably washing the maize plants according to the invention or the maize grains according to the invention before grinding and in
25 addition the step of cultivation of the maize plants according to the invention prior to harvesting.

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In a further embodiment of the present invention, the method according to the invention for production of maize meals (flours) includes processing of the maize plants according to the invention before grinding.

Said processing can, in connection with the production of the grains, for example be
5 heat treatment, preferably thermal treatment and/or drying. The comminution of the maize plants according to the invention, of starch-storing parts or grains of said plants before grinding can also constitute processing in the sense of the present invention. The removal of plant tissue, such as husks of the grains, before grinding also constitutes processing before grinding in the sense of the present invention.

10 In a further embodiment of the present invention, the method according to the invention for production of maize meals (flours) after grinding includes processing of the ground product. The ground product can for instance be sieved after grinding, e.g. in order to produce meals (flours) of various types.

In a further embodiment, the maize meal (= maize flour), or maize starch, according
15 to the invention may be used for the production of a foodstuff.

In a further embodiment, the maize meal (= maize flour), or maize starch, according to the invention may be used as a prebiotic.

In a further embodiment, the present invention relates to a composition containing the maize starch according to the invention and at least one nutritional supplement.

20 In a further embodiment, the present invention relates to a composition containing the maize meal (= maize flour) according to the invention and at least one nutritional supplement.

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- Nutritional supplements are, in connection with the present invention, for example vitamins (e.g. vitamin A, B1, B2, B3, B5, B6, B9, B12, C, D, E, F, K), provitamins, antioxidants, trace elements (e.g. chromium, iron, fluorine, iodine, cobalt, copper, manganese, molybdenum, selenium, vanadium, zinc), minerals (e.g. calcium, chlorine, potassium, magnesium, phosphorus, sulfur, sodium), flavorings, dyes, oils, fats, fatty acids, in particular (poly) unsaturated fatty acids, essential fatty acids, carbohydrates (e.g. starches, galactooligosaccharides, gentiobiose, tagatose), roughage (e.g. cellulose, hemicellulose, pectin, ligin), prebiotics (e.g. oligofructose, oligosaccharides, chitosan, beta glucans, arabinogalactan), probiotics
- 5 (e.g. bifidobacteria, lactic acid bacteria e.g. of the genus *Lactobacillus*),
 10 i.e. nonpathogenic microorganisms, which are added live or as spores to the foodstuffs and can have a beneficial influence on the intestinal flora.

Production of the compositions according to the invention can for example take place by simple mixing.

- 15 In a further embodiment, the present invention relates to a foodstuff containing the maize starch according to the invention.

In a further embodiment, the present invention relates to a foodstuff containing the maize meal (= maize flour) according to the invention.

In a further embodiment, the present invention relates to a foodstuff containing the composition according to the invention.

Typical foodstuffs that can be produced using the maize starch according to the invention, the maize meal (= maize flour) according to the invention or the composition according to the invention, are for example tortillas, tortilla chips, baked goods (e.g. bread, corn bread, rolls, cookies, cakes, waffles, muffins, maize dough-cakes), pancakes, pizza, polenta, pasta products (e.g. noodles), cornmeal mush (USA), porridge (GB), stews, sauces, corn meal pudding, milk products (e.g. yoghurt, quark, ices), puddings, spreads (e.g. butter, margarine), beverages, beverage powders, ready-to-serve meals, (breakfast) cereals, enchilada, sausages, meat products, children's food, ketchup, mayonnaise, barbecue sauces etc.

General methods

Methods that can be used for carrying out the invention described above are described in the following. These methods represent concrete applications of the present invention, but do not restrict the present invention to these methods.

1) Plant material and cultivation

Maize plants: Zea mays, variety A188

Zea mays, mutant ae1 (amylose extender) variety W64A

Wheat plants: Triticum aestivum, variety Fielder

The maize plants are cultivated in the greenhouse in the following conditions:

Substrate: Special sowing mix

80% younger peat

20% black peat

100 kg/m³ sand

40 kg/m³ moist clay

structure: fine

pH value: 5.3 – 6.1

Basal dressing: 2 kg/m³ and 100g/m³ Radigen (Theraflor GmbH; Iserlohn; Germany) (composition: 5% MgO; 2% Fe; 1.5% Cu; 1% Mn; 0.8% Mo; 0.6% B; 0.5% Zn)

Pots: 10L containers
 Spacing: Max. 6 plants/m²
 Watering: Keep plants uniformly moist, but definitely avoid water-logging
 and drying-out of the substrate
 Fertilizer: 1 TAB Plantosan 4g (composition 15% N; 8% P₂O₅; 15% K₂O; 2% MgO
 + trace elements) at the 4-leaf stage
 1 TAB Plantosan after a further 3 weeks
 Temperature: day 22-25°C/night 16°C
 Light: 18 hours, 350-400μEinstein/s/m
 Humidity: 50% rel.

The wheat plants are cultivated in the greenhouse in the following conditions:

Substrate: Special mix
 100% younger peat
 200 L/m³ sand
 180 kg/m³ moist clay
 20% perlite
 structure: medium-coarse
 pH value 5.5 – 6.0
 Basal dressing: 2 kg/m³ 12-14-24 (+3) and 100 mg/m³ Radigen
 (Theraflor GmbH; Iserlohn; Germany) (composition: 5% MgO; 2% Fe;
 1.5% Cu; 1% Mn; 0.8% Mo; 0.6% B; 0.5% Zn)
 Pots: square, volume 1.6 l
 Spacing: 42 pots/m²
 Watering: keep plants uniformly moist, but definitely avoid waterlogging and
 drying-out of the substrate
 Fertilizer: 1g/plant in three doses, Hakaphos (composition 15% N; 10% P₂O₅;
 15% K₂O; 2% MgO) manufacturer Compo
 Temperature: day 20-25°C/night 16°C
 Light: 18 hours, 350-400μEinstein/s/m
 Humidity: rel. 50%

Plant protection according to indication:

Pirimicarb® (Zeneca), Confidor® (Bayer), Neem Azal® (Trifolio-M

GmbH), Vertimec® (Syngenta)

2) Transformation and regeneration of maize and wheat plants

Maize plants were transformed and regenerated according to the method described by Ishida et al. (1996 Nature Biotechnology Vol 14: 745-750).

Transformation and regeneration of the wheat plants were carried out according to the method of Jones et al., (Jones H.D., Doherty A., Wu H., 2005. Review of methodologies and a protocol for the *Agrobacterium*-mediated transformation of wheat. Plant Methods 2005, 1:5 doi:10.1186/1746-4811-1-5).

3) Production and identification of genetically modified maize plants that have an amylose extender mutation and detectable GWD expression

For crossing the transgenic maize plants with detectable GWD expression with the ae1-mutant the variety W64A, the ae1-mutant was put out 7-10 days beforehand and the transgenic lines then correspondingly after reaching the 2-leaf stage of the mutant. This is necessary to synchronize development of the flowers. The ae1-mutant was used as pollen donor (male crossing partner) and the carriers with detectable GWD expression as female crossing partner.

The resultant F1 generation was then self-pollinated and seeds were selected that are homozygous both for the ae1 mutation and for the GWD gene.

4) Production, harvesting and processing of maize grains

For the production of sufficient amounts of study material, maize plants were grown under greenhouse conditions and after reaching complete maturity (*approx. 40 days after pollination*) the cobs were harvested. For further drying, the mature (i.e. fully developed) maize cobs were stored for 3-7 days at 37°C.

After drying (below 13% moisture, measured with the "Grain Moisture Tester Riceter J301" instrument from the company Kett Electric Laboratory, Tokyo, Japan), the grains were removed from the cobs and used as starting material for analyses of the whole grain, for example grain weight.

5) Determination of phosphate content of the starch in position C6 (C6-P content)

In starch, positions C3 and C6 of the glucose units may be phosphorylated. To

determine the C6-P content of starch (modified according to Nielsen et al., 1994, Plant Physiol. 105: 111-117), 50 mg maize meal (flour)/starch was hydrolyzed in 500 ml 0.7 M HCl for 4 h at 95°C, shaking continuously. Then the preparations were centrifuged for 10 min at 15 500 g and the supernatants were purified using a filter membrane (0.45 µm) to remove suspended matter and turbidity. From the clear hydrolyzate, 20 µl was mixed with 180 µl imidazole buffer (300 mM imidazole, pH 7.4; 7.5 mM MgCl₂, 1 mM EDTA and 0.4 mM NADP). Measurement was carried out in a photometer at 340 nm. After determination of basic absorption, the enzyme reaction was started by adding 2 units of glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*, Boehringer Mannheim). The change in absorption is based on equimolar conversion of glucose-6-phosphate and NADP to 6-phosphogluconate and NADPH, with the formation of NADPH being detected at the abovementioned wavelength. The reaction was continued until a plateau was reached. The result of this measurement gives the content of glucose-6-phosphate in the hydrolyzate. From the identical hydrolyzate, the degree of hydrolysis was determined on the basis of the amount of glucose released. Based on the degree of hydrolysis, we are able to relate the content of glucose-6-phosphate to the proportion of hydrolyzed starch in the fresh weight. For this, 10 µl hydrolyzate was neutralized with 10 µl 0.7 M NaOH and then diluted 1:100 with water. 4 µl of this dilution was mixed with 196 µl measuring buffer (100 mM imidazole pH 6.9; 5 mM MgCl₂, 1 mM ATP, 0.4 mM NADP) and used for determining the basic absorption. The reaction was [started] by adding 2 µl enzyme mix (hexokinase 1:10; glucose-6-phosphate dehydrogenase from yeast 1:10 in measuring buffer) and monitored at 340 nm up to the plateau. The measurement principle corresponds to that for the first reaction. The result of this measurement shows the amount of glucose (in mg) that was released in the course of hydrolysis from the starch present in the starting material. Next, the results from the two measurements provide a basis for expressing the content of glucose-6-phosphate per mg of hydrolyzed starch. Instead of relating the amount of glucose-6-phosphate to the fresh weight of the sample, this calculation relates the amount of glucose-6-phosphate only to the portion of the starch that was completely hydrolyzed to glucose and is therefore to be regarded as the source of the glucose-6-phosphate.

6) Determination of swelling power (SP) and solubility in hot water

The swelling power and the solubility in hot water of a 3% (w/v) starch suspension at 90°C were determined by the method of Leach et al. (Cereal Chemistry 36, (1959), 534-544).

For this, the starch suspension was incubated for 30 minutes with continuous shaking (200rpm) at 90°C and, after cooling to room temperature, centrifuged for 15 minutes at 700 x g. The resultant supernatant was removed completely and, for determination of the amount of soluble matter, was dried completely for 48 hours at 37°C and then weighed. The centrifugation residue represented the swollen starch gel, the weight of which is determined gravimetrically.

The following formulas are used:

Swelling power (g/g) = weight of gel / (initial weight of starch – soluble matter)

Solubility in hot water at 90°C in % = weight of dried supernatant / initial weight of starch x 100

7) Determination of apparent amylose content

Determination of apparent amylose content was based on the method of Juliano (1971, Cereal Science Today 16 (10): 334-340).

For each sample, twice 50 mg maize or wheat meal (flour) or maize or wheat starch was weighed in 100 ml Erlenmeyer flasks and moistened successively with 1 ml 95% ethanol and 9 ml 1M NaOH.

In parallel, for construction of a standard curve, flasks with defined amounts of pure amylose were treated in the same way as the meal/starch samples. For this purpose it is possible to use for example maize starch from Sigma-Aldrich (order No. S4126, batch number: #015K0144) which according to manufacturer data has a content of apparent amylose of 27 wt.% and an amylopectin content of 73 wt.%.

For thorough mixing, the flasks were swirled briefly and then incubated for 20 minutes on a boiling water bath, with gentle shaking. After cooling for 5-10 minutes at room temperature (RT) the volume was made up to 100 ml with water.

A 100 µl aliquot was mixed with 1 ml of measuring solution (10 mM acetic acid, 0.004% (w/v) I₂; 0.04% (w/v) KI), stirred well, and the absorption at 620nm was determined against a corresponding blank value. The amylose content was calculated by means of the amylose standards that are used for constructing a calibration curve.

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8) Analysis of maize or wheat starch using the Rapid Visco Analyzer (RVA)

The principle of this analysis is that a suspension of water and maize starch is submitted to a defined temperature and shearing protocol, recording the viscosity of the suspension continuously. The measuring instrument used is an RVA Super3 from the company Newport Scientific (Macclesfield, UK) with the corresponding software "Thermocline for Windows", Version 2.3.

For the analysis, 2.5 g maize/wheat starch (initial weight as pure dry weight of the sample material, corrected to 0% moisture) was weighed in a measuring vessel, 25 ml water was added, and after inserting a stirrer, the measuring vessel was mounted in the equipment.

The following temperature and shearing profile was applied:

Time	Type	Value
00:00:00	Temp	50°C
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:01:00	Temp	50°C
00:04:45	Temp	95°C
00:07:15	Temp	95°C
00:11:00	Temp	50°C
00:12:30	End of test	

At the end of the measurement, the following parameters were determined:

Peak viscosity (highest viscosity between 2 and 8 minutes measuring time)

Trough viscosity (lowest viscosity between 6 and 12 minutes measuring time)

Final viscosity (viscosity at the end of measurement after 12.5 minutes)

Breakdown = peak – trough

Setback = final – trough

Pasting temperature (temperature at which, in a time interval of 0.5 minutes, the viscosity changes by more than 50cP)

Peak time (time at which the peak viscosity is reached)

9) Extraction of maize starch

Extraction of maize starch was based on the method of wet starch extraction described by the Corn Refiners Association, 1701 Pennsylvania Ave., N.W., Suite 950, Washington, DC 20006. 10-50g of maize

grains was weighed and, to disrupt the protein matrix, was incubated in excess with 0.2% sulfurous acid for three days at 50°C. The grains were then washed with water and dried briefly. Comminution was carried out in a Retsch ultracentrifugal mill ZM100 with 2 mm sieve (Retsch, Haan, Germany). The comminuted material was transferred to a beaker, 20% NaCl solution was added, and it was left to stand for at least 30 min. The starch forms a sediment and the lipids float to the surface. The top layer (germs) was poured off and the sediment was resuspended in the remaining supernatant. Then the starch was purified further in several sieving steps. First the sample was passed through a 500µm test sieve (DIN 4188), then through a 200µm Retsch analysis sieve (DIN 4188) and lastly through a 125µm sieve (ISO 3310-1) and then rinsed with NaCl (2-3 l) by means of a pressure sprayer, until the drips under the sieve no longer contained starch. This pre-purified starch was sedimented overnight at room temperature and then the supernatant was poured off apart from approx. 5 mm above the sediment. The starch was transferred to centrifuge tubes and centrifuged in a Heraeus Varifuge (Heraeus, Hanau, Germany) at room temperature at 3500 rpm for 10 min. Then the starch-protein layer at the top (generally of a different color) is scraped off and discarded.

Several washing steps were then carried out, first with 0.2M sodium acetate pH 4.6 (centrifugation as above, 5 min), and after each washing step the starch-protein layer was scraped off again. Then digestion was carried out in 0.2M sodium acetate pH 4.6 with 1% bromelain (from AppliChem, Darmstadt, Germany) and 1% pepsin (from AppliChem, Darmstadt, Germany) and incubated at 37°C for one hour on a Rotator (vertical shaker; Fröbel, Lindau, Germany). It was then centrifuged (see above) and the supernatant was discarded. The starch-protein layer was discarded again and the pellet was resuspended in tap water and centrifuged (see above, 3000 rpm). Once again this was followed by mechanical separation of the protein layer present on the pellet, which was generally clearly demarcated. Another four washing steps with water were carried out, as described above. Then the pellet was washed four times with 80% technical ethanol, and centrifuged (see above, 3000 rpm). Finally it was washed once with acetone, for defatting the starch, and dried at room temperature for two days under an exhaust hood.

As an alternative to the method described above, the starch extraction method described by Eckhoff et al. (Eckhoff, S.R., Rausch, K.D., Fox, E.J., Tso, C.C., Wu, X.,

Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 1993, 70:723-727) was used (see Examples 6 and 7).

10) Detection of branching enzyme activity by means of activity gel

The various branching enzyme activities in unripe maize grains were detected by means of activity gels (zymograms, cf. Fig. 2), in which protein extracts were separated under native conditions in a polyacrylamide gel and then incubated with appropriate substrates. The resultant reaction product (starch) was stained in the gel with Lugol solution (2% (w/v) KI; 0.2% (w/v) I₂).

Individual unripe maize grains (approx. 15 days after flowering – measured from the day when flowering began) were quick-frozen in liquid nitrogen and homogenized in 150-200 µl cold extraction buffer (50 mM Tris/HCl pH 7.6, 2.5 mM EDTA, 2 mM DTT, 4 mM PMSF, 0.1% (w/v) glycogen, 10% (v/v) glycerol). After centrifugation (15 min, 13000 g, 4°C) the clear supernatant was transferred to a fresh reaction vessel and an aliquot of the extract was used for determination of protein content according to Bradford (1976, *Anal Biochem* 72: 248-254).

The protein extracts were separated by means of a continuous 7.5% polyacrylamide gel (7.5% AA/BAA 37.5:1; 25 mM Tris/HCl pH 7.6, 192 mM glycine, 0.1% (w/v) APS, 0.05% (v/v) TEMED) using single-strength running buffer (25 mM Tris/HCl, 192 mM glycine). Before loading the gels, a preliminary run for removal of radicals is carried out for 30 minutes at 8 mA and 4°C. For each sample, 2 µg protein was applied and electrophoresis was carried out for 2-2.5 hours at 8 mA and 4°C. Then the gels were washed five times in washing buffer (0.1M sodium citrate pH 7.0) for 5 minutes on ice. Next, the gels were incubated in 15 ml incubation buffer (0.1M sodium citrate pH 7.0, 50 mM glucose-1-phosphate, 1 mM AMP, 0.95Units/ml phosphorylase A from rabbit) overnight at room temperature, shaking continuously. The starch that formed was stained with Lugol solution.

11) Detection of the expression of GWD from *Curcuma longa* by quantitative PCR

RNA was prepared from leaf samples that were frozen in liquid nitrogen directly after sample collection. After homogenization with a 4 mm steel ball (Retsch mill, 30 Hz,

45s) the RNA was prepared with the "SV 96 Total RNA Isolation System" from Promega according to protocol No. 294 (Promega). The RNA was treated in each case with 10 µl "RQ1 RNase-free DNase" (Promega) according to the manufacturer's instructions.

Quantitative RT-PCR was carried out with the reagents of the "Access RT-PCR System" from Promega. The amplicon for R1 from *Curcuma longa* had a length of 105 bp (base pairs). Cl_R1-F1 (TggATAAATACAAAgAgTgAAgCAg = SEQ ID No. 11) and Cl_R1-R1 (ggACATTgAAggTgTTgTAAAgg = SEQ ID No. 12) were used as amplification primers. The doubly fluorescence-labeled oligonucleotide Cl_R1-FAM (FAM- cttcgtcgtccaaacaagaccacag –TAMRA = SEQ ID NO. 13) was used as Taqman probe.

The amplicon for catalase from maize had a length of 73 base pairs. The primers Zm_Cat-F1 (GTGGGAGCAACTCCAGCTT = SEQ ID No. 14), Zm_Cat-R1 (CGGTGAGGGCAGAGTTGTT= SEQ ID No. 15) and the doubly fluorescence-labeled TaqMan probe Cat-VIC (VIC-ACTCCGGCGCCCCCGT –TAMRA 0 SEQ ID No. 16) were used for the amplification.

The 30-µl reaction mixture contained buffer (singly), 3 mM MgSO₄, in each case 500 nM amplification primer, 100 nM probe Cl_R1-FAM or Cat-VIC, in each case 0.2 mM deoxy-ribonucleotides, in each case 0.6 µl reverse transcriptase and Tfl-polymerase (corresponding to protocol 294). Each mixture contained 350 ng total RNA.

The reaction conditions for the RT-PCR were: 30 min 55°C, 2 min 94°C, 40 x (15s 94°C, 1 min 60°C). The fluorescence signal was recorded by the ABI Prism 7700 instrument (Applied Biosystems) in each case during the combined annealing/extension phase.

In each case, mixtures without reverse transcriptase in the mixture were conducted in parallel, as controls.

The relative expression was calculated according to M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Research 2001, Vol. 29, No. 9 00. The expression of R1 from *Curcuma longa* was standardized to the expression of catalase.

12) Determination of the copy number for GWD from *Curcuma* by PCR

DNA was prepared from leaf samples that were frozen in liquid nitrogen directly after sample collection. After homogenization with a 4 mm steel ball (Retsch mill, 30 Hz,

45s) it was prepared with the Qiagen Kit "DNeasy plant Mini kit". Quantitative PCR was carried out with the following amplicons:

The amplicon for R1 from *Curcuma longa* had a length of 105 bp (base pairs). Cl_R1-F1 (TggATAAATACAAAgAgTgAAgCAg = SEQ ID No. 11) and Cl_R1-R1 (ggACATTgAAggTgTTgTAAAgg = SEQ ID No. 12) were used as amplification primers. The doubly fluorescence-labeled oligonucleotide Cl_R1-FAM (FAM-cttcgtcgtccaaacaagaccacag –TAMRA = SEQ ID NO. 13) was used as Taqman probe.

The amplicon for catalase from maize had a length of 73 base pairs. The primers Zm_Cat-F1 (GTGGGAGCAACTCCAGCTT=SEQ ID No. 14), Zm_Cat-R1 (CGGTGAGGGCAGAGTTGTT= SEQ ID No. 15) and the doubly fluorescence-labeled TaqMan probe Cat-VIC (VIC-ACTCCGGCGCCCCCGT –TAMRA = SEQ ID No. 16) were used for the amplification.

The HotStar Mastermix from Qiagen was used for the reactions (30 µl). The reaction mixture contained Mastermix (singly) for which the final concentration of $MgCl^{2+}$ was adjusted to 3 mM. The final concentrations of the amplification primers were in each case 300 nM and that of the probe Cl_R1-FAM or Cat-VIC was 100 nM. According to the manufacturer's data the final concentration of the deoxy-nucleotides was 200 µM and the enzyme concentration was 1.5 units Taq-polymerase/per 30-µl preparation. Two preparations were analyzed in each case with 50 ng or 5 ng DNA as template per preparation.

The reaction conditions for the PCR were: 2 min 94°C, 40 x (15s 94°C, 1 min 60°C). The fluorescence signal was recorded with the ABI Prism 7700 instrument (Applied Biosystems) in each case during the combined annealing/extension phase.

The relative copy numbers were calculated according to M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Research 2001, Vol. 29, No. 9 00. The amount of DNA was standardized on the basis of the amplification signal for catalase.

13) Determination of light transmittance

To determine the light transmittance of a starch sample the method of Craig et al. (Cereal Chemistry 66(3), (1989), 173-182, see page 173, column 2 "Light Transmittance (%T) of Starch Pastes) is used. For this, 50 mg of starch (dry weight) are mixed with 5 ml of water and subsequently heated in a boiling water bath for

30 minutes during which the samples are carefully shaken every 5 minutes. The sample is then cooled to room temperature within 5 minutes and the light transmittance (in %) compared with a water sample is determined at 650 nm in a spectrophotometer.

14) Determination of freeze-thaw stability

Freeze-thaw stability is determined in line with the method described by Yuan & Thompson (Cereal Chemistry, 1998, 75 (4) 571-573). A 5% (w/w) starch solution is heated to 88°C and incubated at 88°C for 10 minutes. After the sample has cooled to room temperature, about 4 ml are transferred into a 5 ml vessel of defined weight. The weight of the vessel including its contents (starch suspension) is then determined.

Two vessels are filled for each starch sample. One sample is immediately centrifuged at 5000 rpm for 10 minutes, while the other sample is frozen at -18°C. The aqueous supernatant of the centrifuged sample is removed by means of a Pasteur pipette and the weight of the vessel containing the starch paste is determined. The water loss of the control sample in % is obtained from the weight of the sample after centrifuging and decanting (pipette), divided by the weight of the paste before centrifuging multiplied by 100.

For the subsequent freeze-thaw cycle, the vessels are taken from the freezer compartment and thawed at room temperature (4 hours). The thawed samples are likewise centrifuged at 5000 rpm for 10 minutes and, to determine the water loss, treated like the control samples.

15) Brabender analysis of maize or wheat starch by means of a Brabender visco-amylograph

The principle of the analysis corresponds to that of method 8, but differs with regard to the sample quantity and concentration used and also the analysis time.

The instrument used is a Micro Visco-Amylo-Graph from Brabender GmbH & Co. KG, Duisburg, Germany.

A 5.5% (w/w) starch suspension (total weight 110 g) is heated to 50°C at the start of analysis and subsequently, with continuous stirring at 250 rpm, heated to 95°C at a heating rate of 1.5°C/min. After 30 minutes at 95°C, the sample is cooled back down to 50°C at a cooling rate of 1.5°C/min, and is incubated at 50°C for a further

30 minutes. The viscosity profiles are evaluated automatically by means of the following software: Micro Visco AG, WinVis-2.4.7, VisCorr-2.1.2, Univ.Eval-1.1.2.

16) Determination of RS and RDS

Determination of RS, SDS and RDS is done via the method of Englyst et al. (Europ. J. of Clinical Nutrition 46 (Suppl. 2), (1992), S 33-50, see in particular the following sections from Englyst et al., page S35-S36: "Reagents, Apparatus, Spectrophotometer"; page S36-S37, paragraph "Measurement of free glucose (FG)"; page S38, paragraph "Measurement of RDS and SDS"; S39 for RS determination: measurement of RS₃).

Example 1

Production of a construct for the transformation of maize plants that display detectable GWD gene expression

The pMZ12 plasmid served as the starting plasmid for production of the pSC16 plasmid, which was used for the transformation of maize plants. This plasmid contains the *ColE1* origin of the pBR322 plasmid (Bolivar et al., 1977, Gene 2, 95-113) and a bacterial selection marker, which imparts resistance to the antibiotic gentamicin (Wohlleben et al., 1989, MGG 217, 202-208). In addition, this plasmid contains a right and a left T-DNA border sequence. Between these T-DNA border sequences, the plasmid contains a *bar* gene from *Streptomyces hygroscopicus* (White et al., 1990, NAR 18, 1062; EMBL Acc.: X17220), which imparts resistance to the herbicide glufosinate. Expression of the *bar* gene is initiated by the promoter of the *actin* gene from rice (McElroy et al., 1990, Plant Cell 2, 163-171). For stabilization of expression of the *bar* gene, the 1st intron of the *actin* gene from rice (McElroy et al., 1990, Plant Cell 2, 163-171) is inserted between the *actin* promoter and the sequence encoding the *bar* protein. The sequence encoding the *bar* protein is followed by the polyadenylation signal of the nopaline synthase gene from *Agrobacterium tumefaciens* (Depicker et al., 1982, J Mol. Appl. Gent. 1, 561-573).

The ubiquitin promoter from *Zea mays* (Christensen et al. 1992, Plant Mol. Bio 18, 675-689) was inserted in the pMZ12 plasmid, followed by the 1st intron of the ubiquitin gene from *Zea mays* (Christensen et al. 1992, Plant Mol. Bio 18, 675-689),

followed by the coding sequence of the GWD (R1) gene from *Curcuma longa* (see SEQ ID NO 3) with the aid of the Gateway system from Invitrogen and so-called attB recognition sequences (Hartley J.L., Temple G.F., Brasch M.A. (2000). DNA cloning using in vitro site-specific recombination. *Genome Research*, 10, 1788-1795), followed by the polyadenylation signal of the nopaline synthase gene from *Agrobacterium tumefaciens* (Depicker et al., 1982, *J Mol. Appl. Gent.* 1, 561-573) in addition to the selection cassette between the left and right T-DNA border sequence. The resultant plasmid was designated as pSC16.

Example 2

Production and analysis of maize plants that have detectable GWD expression

Ten days after pollination, immature embryos of maize plants (variety A188) were isolated and were transformed according to the method described in Ishida et al. (1996, *Nature Biotechnology* 14, 745-750), using *Agrobacterium tumefaciens*, containing the pSC16 plasmid as cointegrate. So-called T0 plants resulting from this transformation were grown in the greenhouse. The plants obtained were given the designation SC16-X, where X denotes independent plants resulting from the transformation.

Maize plants (T0 plants) resulting from transformation with the expression vector pSC16 were cultivated in soil in the greenhouse and were pollinated with wild-type (variety A188) pollen. Meal (flour) was produced from individual, ripe grains (T1 seeds). For this, individual grains were comminuted in a ball mill (from Retsch, Haan, Germany, model MM300) for 30 seconds at a frequency of 30 Hz. Then the starch phosphate content in position C6 of glucose molecules of the starch was determined, as described under general methods.

For selected plants, the following results were obtained:

Designation of the plant	nmol C6-phosphate per mg starch
SC16-1	1.9
SC16-2	1.4
SC16-3	1.6
WT A188	0.1

As can be seen from the table, independent lines could be identified, which have an

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increased phosphate content in position C6 of the glucose monomers of the starch compared with corresponding wild-type plants (A188) that are not genetically modified. The increase in phosphate content in lines with the designation pSC16 can be attributed to the detectable expression of a GWD gene from *Curcuma longa* (SEQ ID No. 3).

Example 3

Production of plants that have both the amylose extender character and detectable expression of a glucan-water dikinase

In each case 30 T1 seeds from plants of various lines with the designation SC16-1-X, which have detectable expression of a GWD gene from *Curcuma longa* compared with the wild-type maize plants of variety A188, were cultivated in the greenhouse and the resultant plants were sprayed with 0.5% Basta® (Bayer CropScience) solution. About half the treated plants of the pSC16-1-X lines reacted sensitively to the treatment with Basta®, so it could be concluded that they did not contain a bar gene imparting resistance to Basta®. For the resistant plants it could accordingly be concluded that the T-DANN(s) was integrated at one place in the genome or were integrated at places in the genome that were so close together that they do not segregate. These plants were self-pollinated and the resultant T1S1 seeds from T1 plants, which were resistant to treatment with Basta®, were again sown in the greenhouse, treated with Basta® as just described, self-pollinated and resultant T1S2 seeds were produced. Then the same treatment with Basta® was carried out with T1S2 plants of these lines: various T1S2 plants were identified, for which all descendants were resistant to Basta®. It could therefore be concluded that the starting T1S2 plants were homozygous for the integrated T-DNA. T1S2 seeds from homozygous plants of line SC16-1 were again sown and various plants were in each case pollinated with pollen of the amylose-extender mutant ae1 of variety W64A (cf. General Methods), which was obtained from the Maize Genetics Cooperation Stock Center at the University of Illinois, Urban/Champaign, S-123 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801-4730, USA. The resultant cross-bred descendants were designated SC16 x ae1.

Example 4

Analysis of plants that have both the amylose extender character and detectable expression of a glucan-water dikinase

From the SC16 x ae1 plants resulting from the crossing described in Example 3, in each case F1 seeds were harvested, sown again and self-pollinated. F1S1 seeds and plants from F1 plants of this crossing were investigated with respect to the copy number of the inserted GWD gene from *Curcuma longa* and the amylose content. Seeds that had an amylose content of at least 40% (see General Methods) were cultivated and the F1S1 plants, which then contained a copy number of 2 for the GWD transgene from Curcuma (see General Methods), were used for the production of doubly homozygous F1S2 seeds in the greenhouse. The reduced branching enzyme activity was detected with the aid of activity gels (see Fig. 2 and "General methods"):

Designation of the plant	No. of the homozygous seed	% amylose
SC16 x ae1	1	51.1
	2	49.4
	3	45.9
	4	49.8
	5	52.4
SC 16 -1 (mother)	1	25.5
	2	26.0
ae1 (father)	1	52.4
	2	51.9
WT A188	1	26.0
	2	26.5

Designation of the plant	No. of the homozygous plant	Copy number
SC16 x ae1	1	2
	2	2
	3	2
	4	2
	5	2
SC 16 -1 (mother)	1	2
	2	2
ae1 (father)	1	0
	2	0
WT A188	1	0
	2	0

Example 5

Analysis of the maize starch from maize grains from doubly homozygous plants of the crossing SC16 x ae1

1. Amylose content

The amylose content of starches produced from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and of the wild-type variety A188 was determined as described under General Methods. The following results were obtained:

Designation of the plant	Amylose (% dry weight)
SC16 x ae1	43.1
SC 16	25.0
ae1	52.9
WT A188	27.0

2. Analysis of the viscosity properties of maize starch using the Rapid Visco Analyzer (RVA)

The viscosity properties of 10% (w/v) suspensions of various maize starches were investigated using the method "Analysis of maize meal (flour) by RVA" (AACC Method 61-02):

Sample:	Wild-type (A188)	SC16	ae1	SC16 x ae1	Amylogel®	Hylon ® VII
Peak viscosity (cP)	1844	2069	2	1594	40	2
Trough (cP)	329	525	0	1517	32	0
Breakdown (cP)	1515	1544	2	77	8	2
Final viscosity (cP) (12.5 min)	705	1088	0	1580	42	0
Setback (cP) (12.5 minutes)	376	563	0	63	10	0
Peak time (min)	3.94	3.94	2.02	6.55	6.58	2.02
Pasting temperature (°C)	73.25	72.35	Error	85.35	Error	Error

The results show a greatly altered RVA profile (see Fig. 1) of the starch according to the invention of the crossing SC16 x ae1 compared with the parent lines, mainly with amylose extender (ae1), and compared with conventional high-amylose maize starch (Amylogel®, Hylon® VII). The starch of the amylose-extender line and the commercially available high-amylose maize starches (Amylogel®, Hylon® VII) do not undergo gelatinization to a significant extent, accordingly no pasting temperature can be determined in RVA analysis (ERROR). The starch sample of the crossing SC16 x ae1 undergoes gelatinization at a much higher temperature than the SC16 or wild-type starch. The final viscosity of the starch according to the invention is greater than for SC16 or wild-type starches and shows a very low setback.

3. Swelling power and solubility in hot water

The swelling power and the solubility in hot water of starches produced from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and from wild-type plants were determined as described under General Methods. The following results were obtained:

Designation of the plant	Swelling power of starch [g/g]	Solubility in %
SC16 x ae1	12.6	12.4
SC 16	27.2	7.1
ae1	5.4	6.9
WT A188	23.2	20.9
Hylon VII ®	5.0	6.4
Amylogel®	5.2	5.6

The results represent the mean value from two independent measurements. They show that both the starch of the amylose-extender mutant (ae) and the commercial products (Hylon VII ®, Amylogel®) have a far lower swelling power and a far lower solubility than the starches according to the invention of the crossing SC16 x ae1.

4. Phosphate content in position C6 of the glucose monomers of starch

The C6-phosphate content of starches produced from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and from the wild-type variety A188 was determined as described under General Methods. The following results (mean value of two measurements) were obtained:

Designation of the plant	nmol C6-phosphate/mg starch
SC16 x ae1	20.9
SC 16 -1 (mother)	4.3
ae1 (father)	1.0
WT A188	0.1

Overall, the combination SC16 x ae1 shows a significant increase in phosphate content in position C6.

Example 6

Analysis of the maize starch from maize grains from doubly homozygous plants of the crossing SC16 x ae1 kept in the open

The SC16 x ae1 maize plants described in Examples 4 and 5 were kept in the open and harvested after about 4 months. After the harvest, starch was extracted by means of the method of Eckhoff et al. (Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. Cereal Chem. 1993, 70:723-727) and subsequently analyzed.

1. Amylose content

The amylose content of starches prepared from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and from the wild-type variety A188 was determined as described under General Methods. The following results were obtained:

Designation of the plant	Amylose (% dry weight)
SC16 x ae1	54.8
SC 16	29.3
ae1	67.5
WT A188	31.4

2. Analysis of the viscosity properties of maize starch using the Rapid Visco Analyzer (RVA)

The viscosity properties of 10% (w/v) suspensions of various maize starches were

investigated by means of the method "Analysis of maize flour by RVA" (AACC method 61-02):

Sample:	ae1	SC16 x ae1
Peak viscosity (cP)	0	2249
Trough (cP)	0	2073
Final viscosity (cP) (12.5 min)	0	2334
Setback (cP) (12.5 minutes)	0	261
Peak Time (min)	0	6.42
Pasting temperature (°C)	Error	85.35

The results show a distinctly modified RVA profile for the starch of the crossing SC16 x ae1 according to the invention compared with amylose extender (ae1). The starch of the amylose extender line does not gelatinize, so that it is also impossible to determine a pasting temperature in RVA analysis (ERROR).

3. Swelling power

The swelling power of starches, prepared from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and from wild-type plants was determined as described under General Methods. The following results were obtained:

Designation of the plant	Swelling power of starch [g/g]
SC16 x ae1	15.2
SC 16	21.8
ae1	5.5
WT A188	22.4

The results represent the rounded mean of two independent measurements. They show that the present invention starch of the crossing SC16 x ae1 has distinctly increased swelling power compared with the starch of the amylose extender mutant (ae1).

4. Phosphate content at in position C6 of the glucose monomers of starch

The C6-phosphate content of starches produced from doubly homozygous seeds of the crossing SC16 x ae1, the parent lines and from the wild-type variety A188 was determined as described under General Methods. The following results (mean value of two measurements) were obtained:

Designation of the plant	nmol C6-phosphate / mg starch
SC16 x ae1	21.3
SC 16 -1 (mother)	5.5
ae1 (father)	1.1
WT A188	0.3

Overall, the combination SC16 x ae1 shows a significant increase in phosphate content in position C6.

5. Brabender analysis (see also Figure 3)

Designation of the plant	Pasting temperature in °C	Peak viscosity in Brabender units (BUs)	Final viscosity in BUs
SC16 x ae1	90.8	100	89
ae1 (father)	Not determinable	29	24
WT A188	83.4	244	254
Hylon VII ®	Not determinable	28	21

6. Freeze-thaw stability

Designation of the plant	% water loss after one freeze-thaw cycle
SC16 x ae1	63
ae1 (father)	77
WT A188	41

7. Light transmittance of starch pastes

Designation of the plant	Light transmittance at 650 nm
SC16 x ae1	12.3
ae1 (father)	3.4
WT A188	20.8

8. RDS content of native maize starch

Designation of the plant	RDS content in %
WT A188	30.1
SC16	37.2
ae1 (father)	13.8
SC16 x ae1	10.1

9. RDS content of processed starch compared with RDS content of correspondingly processed starch from A188 maize wild-type plants (100%)

	RVA ¹	Hot-moist treatment ²	Miniloaf ³
A188	100.0	100.0	100.0
SC16 (GWD)	98.7	142.5	n.d.
SC16 x ae1	88.6	64.5	64.0
ae1 (father)	88.8	73.6	76.0

¹ **RVA** = A 10% (w/w) starch suspension was heated up and cooled back down with constant stirring, as described in AACC method 61-02. The resulting gel was left covered at room temperature for 24 hours before aliquots for determining digestibility (RDS, SDS and RS) were taken.

² **Hot-moist treatment** = A 50% (w/w) starch-water mixture was covered and incubated in a water bath at 95°C for 30 minutes. The hot-moist treated starch was subsequently stored at 4°C for two days before the batch was used to determine digestibility (RDS, SDS, RS).

³ **Miniloaves** = A mixture of 80% (w/w) starch, 17% (w/w) wheat gluten and 3% (w/w) water-soluble wheat constituents was prepared. The weight of this mixture being regarded as 100%, the following were also added: 2% (w/w) salt, 1% (w/w) sugar, 4% (w/w) yeast and 5% (w/w) shortening. The ingredients were mixed for one minute before 60% (w/w) water was added and the mixture was mixed and kneaded for a further five minutes. After resting for 10 minutes, aliquots of the minidough thus produced were introduced into DSC crucibles and hermetically sealed. The baking simulation in the DSC instrument started at a temperature of 10°C and was raised to 110°C at a heating rate of 10°C/minute. Subsequently, the DSC crucibles were cooled down to 20°C at a temperature rate of 10°C/minute. The resulting miniloaves

were stored at room temperature for three days before the crucibles were opened and the miniloaves were used to determine digestibility (RDS, SDS, RS).

Example 7

Production and analysis of plants that have both the amylose extender character and detectable expression of a glucan-water dikinase from *Solanum tuberosum*

Standard methods were used to create in the background variety A188, by agrobacterium transformation, transgenic maize plants having a detectable expression of a GWD gene from *Solanum tuberosum* (see SEQ ID No.1) compared with the A188 maize wild-type plants. These were repeatedly self-pollinated to produce plants which were homozygous with regard to the GWD gene introduced. Seeds of these homozygous plants, which were designated as HN3, were again sown and each pollinated with pollen from the amylose extender mutant ae1 of variety W64A. The resulting crossing descendants were designated HN3 x ae1.

These were self-pollinated and used to produce doubly homozygous plants, i.e. maize plants which were homozygous not only for the GWD gene introduced but also for the amylose extender mutation.

Then, maize starch was extracted by the method of Eckhoff et al. (Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 1993, 70:723-727) and then the amylose content, the C6 phosphate content, the swelling power and the viscosity properties in the Brabender were analyzed.

1. Amylose content

The amylose content of starches produced from doubly homozygous seeds of the crossing HN3 x ae1, the parent lines and the wild-type variety A188 was determined as described under General Methods. The following results were obtained:

Designation of the plant	Amylose (% dry weight)
HN3 x ae1	42.0
HN3	25.3
ae1	50.2
WT A188	25.8

2. Swelling power

The swelling power and the hot water solubility of starches produced from doubly homozygous seeds of the crossing HN3 x ae1, the parent lines and from wild-type plants were determined as described under General Methods. The following results were obtained:

Designation of the plant	Swelling power of starch [g/g]
HN3 x ae1	11.63
ae1	5.8
WT A188	22.2

The results represent the mean value of two independent measurements. They show that the present invention starch of the crossing HN3 x ae1 has a distinctly increased swelling power compared with the starch from the amylose extender mutant (ae).

3. Phosphate content in position C6 of the glucose monomers of starch

The C6-phosphate content of starches produced from doubly homozygous seeds of the crossing HN3 x ae1, the parent lines and from wild-type variety A188 was determined as described under General Methods. The following results (mean value of two measurements) were obtained:

Designation of the plant	nmol C6-phosphate / mg starch
HN3 x ae1	29.6
HN3 -1 (mother)	5.5
ae1 (father)	0.7
WT A188	0.2

Overall, the combination HN3 x ae1 shows a significant increase of the phosphate content in position C6.

4. Analysis of the viscosity properties of maize starch by Rapid Visco Analyzer (RVA)

The viscosity properties of 10% (w/v) suspensions of various maize starches were investigated by means of the method "Analysis of maize flour by RVA" (AACC method 61-02). The peak viscosity of the starch from two selected lines of the crossing HN3 x ae1 was 1698 centipoise (compared with the 30 centipoise of the ae1 mutant of the variety W64A), the final viscosity after 12.5 minutes was 1959 or 2164 centipoise (compared with the 5 centipoise of the ae1 mutant of the variety W64A),

30725-968PPH

the trough viscosity was 1586 or 1611 centipoise (compared with the 4 centipoise of the ae1 mutant of the variety W64A) and the pasting temperature was 82.75°C or 83.20°C (compared with "error" in the case of the ae1 mutant of the variety W64A, i.e. not determinable using the AACC method).

5 BRIEF SUMMARY OF SEQUENCES

SEQ ID NOs 1 and 2 represent a nucleic acid sequence and its corresponding amino acid sequence, encoding a protein with the activity of a glucan water dikinase from *Solanum tuberosum*.

10 SEQ ID NOs 3 and 4 represent a nucleic acid sequence and its corresponding amino acid sequence, encoding a protein with the activity of a glucan water dikinase from *Curcuma longa*.

SEQ ID NOs 5 and 6 represent a nucleic acid sequence and its corresponding amino acid sequence, encoding an "amylose extender" gene (also "BEIIb" gene) from maize.

15 SEQ ID NOs 7 and 8 represent a nucleic acid sequence and its corresponding amino acid sequence, encoding the "BEIIa" gene from wheat.

SEQ ID NOs 9 and 10 represent a nucleic acid sequence and its corresponding amino acid sequence, encoding the "BEIIb" gene from wheat.

20 SEQ ID NOs 11 and 12 represent amplification primer sequences for *Curcuma longa* and SEQ ID NO 13 represents the sequence of the doubly fluorescence-labeled oligonucleotide TaqMan probe.

SEQ ID NOs 14 and 15 represent amplification primer sequences for catalase from maize and SEQ ID NO 16 represents the sequence of the doubly fluorescence-labeled oligonucleotide TaqMan probe Cat-VIC.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 30667-31 Seq 22-09-09 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

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<120> Starches with high amylose content and improved processing properties

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1

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Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp Gln Ile Ile	
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Ser Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp Glu Leu	
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Leu Ser Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu Val	
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Ala Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val	
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Ala Leu Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser	
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Val Arg Ala Arg Asn Gly Lys Val Cys Phe Ala Thr Cys Phe Asp	
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Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala Lys Glu Leu Gln	
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Gly Arg Ala Leu Ser Phe Ile Cys Lys Lys Lys Asp Leu Asn Ser	
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Gly Asp Ile Ser Thr Lys Val Leu Lys Gln Leu Lys Val Glu Lys Tyr	
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Phe Ser Asn Lys Arg Ile Gln Arg Lys Glu Arg Asp Ile Met Glu Ile	
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Leu Asn Lys Lys Val Ala Glu Thr Leu Asp Glu Lys Ser Ser Gln Ile	
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Val Thr Pro Pro Thr Val Leu Glu Leu Leu Ala Lys Ser Ile His Glu	
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Gln Asp Gly Glu Ser Val Leu His Gln Lys Ile Tyr Lys Leu Asp Asn	
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Lys Asn Leu Leu Val Leu Val Thr Lys Pro Phe Glu Arg Thr Lys Val	
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Ser Arg Lys Ser Arg Glu Trp Met Val Pro Pro Thr Ser Ser Ile Pro	
485 490 495	

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Gly Leu Met Val Asp Gln Tyr Tyr Gln Ala Ile Gln Ile Glu Ile Asp	
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Leu Asp Lys Ile Ala Asp Leu Glu Thr Glu Ala Gln Lys Ser Phe Met	
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His Arg Phe Ser Ile Ala Ala Asp Leu Thr Glu Gln Ala Arg Gly Ser	
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Lys Asp Phe Pro Gln Tyr Arg Glu Ile Leu Arg Met Ile Met Ala Thr	
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94

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<210> 6
 <211> 799
 <212> PRT
 <213> Zea mays

<400> 6
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Gly	Ala	Met	Arg	Ala	Ala	Ala	Ala	Ala	Arg	Lys	Ala	Val	Met	Val	Pro
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Glu	Gly	Glu	Asn	Asp	Gly	Leu	Ala	Ser	Arg	Ala	Asp	Ser	Ala	Gln	Phe
65					70					75					80
Gln	Ser	Asp	Glu	Leu	Glu	Val	Pro	Asp	Ile	Ser	Glu	Glu	Thr	Thr	Cys
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Gly	Ala	Gly	Val	Ala	Asp	Ala	Gln	Ala	Leu	Asn	Arg	Val	Arg	Val	Val
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Pro	Pro	Pro	Ser	Asp	Gly	Gln	Lys	Ile	Phe	Gln	Ile	Asp	Pro	Met	Leu
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Gln	Gly	Tyr	Lys	Tyr	His	Leu	Glu	Tyr	Arg	Tyr	Ser	Leu	Tyr	Arg	Arg
	130					135					140				
Ile	Arg	Ser	Asp	Ile	Asp	Glu	His	Glu	Gly	Gly	Leu	Glu	Ala	Phe	Ser
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Arg	Ser	Tyr	Glu	Lys	Phe	Gly	Phe	Asn	Arg	Ser	Ala	Glu	Gly	Ile	Thr
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Tyr	Arg	Glu	Trp	Ala	Pro	Gly	Ala	Phe	Ser	Ala	Ala	Leu	Val	Gly	Asp
			180					185					190		
Phe	Asn	Asn	Trp	Asp	Pro	Asn	Ala	Asp	Arg	Met	Ser	Lys	Asn	Glu	Phe
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Gly	Glu	Ile	Pro	Tyr	Asp	Gly	Ile	Tyr	Tyr	Asp	Pro	Pro	Glu	Glu	Val
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Lys	Tyr	Val	Phe	Arg	His	Ala	Gln	Pro	Lys	Arg	Pro	Lys	Ser	Leu	Arg
		275					280					285			
Ile	Tyr	Glu	Thr	His	Val	Gly	Met	Ser	Ser	Pro	Glu	Pro	Lys	Ile	Asn
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Gly	Tyr	Asn	Ala	Val	Gln	Ile	Met	Ala	Ile	Gln	Glu	His	Ser	Tyr	Tyr
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Gly	Ser	Phe	Gly	Tyr	His	Val	Thr	Asn	Phe	Phe	Ala	Pro	Ser	Ser	Arg
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Phe	Gly	Thr	Pro	Glu	Glu	Leu	Lys	Ser	Leu	Ile	Asp	Arg	Ala	His	Glu
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Leu	Gly	Leu	Leu	Val	Leu	Met	Asp	Val	Val	His	Ser	His	Ala	Ser	Ser
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Phe	His	Ser	Gly	Pro	Arg	Gly	His	His	Trp	Met	Trp	Asp	Ser	Arg	Leu
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Phe	Asn	Tyr	Gly	Asn	Trp	Glu	Val	Leu	Arg	Phe	Leu	Leu	Ser	Asn	Ala
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Val	Tyr	Leu	Met	Leu	Val	Asn	Asp	Leu	Ile	His	Gly	Leu	Tyr	Pro	Glu
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Ala	Val	Thr	Ile	Gly	Glu	Asp	Val	Ser	Gly	Met	Pro	Thr	Phe	Ala	Leu
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Pro	Val	His	Asp	Gly	Gly	Val	Gly	Phe	Asp	Tyr	Arg	Met	His	Met	Ala
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Val	Ala	Asp	Lys	Trp	Ile	Asp	Leu	Leu	Lys	Gln	Ser	Asp	Glu	Thr	Trp
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Lys	Met	Gly	Asp	Ile	Val	His	Thr	Leu	Thr	Asn	Arg	Arg	Trp	Leu	Glu	545	550	555	560
Lys	Cys	Val	Thr	Tyr	Ala	Glu	Ser	His	Asp	Gln	Ala	Leu	Val	Gly	Asp	565	570	575	
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Ala	Leu	Asp	Arg	Pro	Ser	Thr	Pro	Thr	Ile	Asp	Arg	Gly	Ile	Ala	Leu	595	600	605	
His	Lys	Met	Ile	Arg	Leu	Ile	Thr	Met	Gly	Leu	Gly	Gly	Glu	Gly	Tyr	610	615	620	
Leu	Asn	Phe	Met	Gly	Asn	Glu	Phe	Gly	His	Pro	Glu	Trp	Ile	Asp	Phe	625	630	635	640
Pro	Arg	Gly	Pro	Gln	Arg	Leu	Pro	Ser	Gly	Lys	Phe	Ile	Pro	Gly	Asn	645	650	655	
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His	Leu	Glu	Gln	Lys	Tyr	Glu	Phe	Met	Thr	Ser	Asp	His	Gln	Tyr	Ile	690	695	700	
Ser	Arg	Lys	His	Glu	Glu	Asp	Lys	Val	Ile	Val	Phe	Glu	Lys	Gly	Asp	705	710	715	720
Leu	Val	Phe	Val	Phe	Asn	Phe	His	Cys	Asn	Asn	Ser	Tyr	Phe	Asp	Tyr	725	730	735	
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His	Phe	Thr	Ala	Asp	Cys	Ser	His	Asp	Asn	Arg	Pro	Tyr	Ser	Phe	Ser	770	775	780	
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<210> 7
 <211> 2310
 <212> DNA
 <213> Triticum aestivum

<400> 7

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<210> 8
<211> 769
<212> PRT
<213> Triticum aestivum

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35          40          45
Leu Glu Ser Ser Glu Pro Thr Gln Gly Ile Val Glu Thr Ile Thr Asp
50          55          60
Gly Val Thr Lys Gly Val Lys Glu Leu Val Val Gly Glu Lys Pro Arg
65          70          75          80
Val Val Pro Lys Pro Gly Asp Gly Gln Lys Ile Tyr Glu Ile Asp Pro
85          90          95
Thr Leu Lys Asp Phe Arg Ser His Leu Asp Tyr Arg Tyr Ser Glu Tyr
100         105         110
Arg Arg Ile Arg Ala Ala Ile Asp Gln His Glu Gly Gly Leu Glu Ala
115         120         125
Phe Ser Arg Gly Tyr Glu Lys Leu Gly Phe Thr Arg Ser Ala Glu Gly
130         135         140
Ile Thr Tyr Arg Glu Trp Ala Pro Gly Ala His Ser Ala Ala Leu Val
145         150         155         160
Gly Asp Phe Asn Asn Trp Asn Pro Asn Ala Asp Thr Met Thr Arg Asp
165         170         175
Asp Tyr Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Ala Asp Gly Ser
180         185         190
Pro Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro
195         200         205
Ser Gly Val Lys Asp Ser Ile Ser Ala Trp Ile Lys Phe Ser Val Gln
210         215         220
Ala Pro Gly Glu Ile Pro Phe Asn Gly Ile Tyr Tyr Asp Pro Pro Glu
225         230         235         240
Glu Glu Lys Tyr Val Phe Gln His Pro Gln Pro Lys Arg Pro Glu Ser
245         250         255
Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser Pro Glu Pro Lys
260         265         270
Ile Asn Ser Tyr Ala Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys
275         280         285
Arg Leu Gly Tyr Asn Ala Val Gln Ile Met Ala Ile Gln Glu His Ser
290         295         300
Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser
305         310         315         320

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Ser Arg Phe Gly Thr Pro Glu Asp Leu Lys Ser Leu Ile Asp Arg Ala
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 His Glu Leu Gly Leu Leu Val Leu Met Asp Ile Val His Ser His Ser
 340 345 350
 Ser Asn Asn Thr Leu Asp Gly Leu Asn Gly Phe Asp Gly Thr Asp Thr
 355 360 365
 His Tyr Phe His Gly Gly Pro Arg Gly His His Trp Met Trp Asp Ser
 370 375 380
 Arg Leu Phe Asn Tyr Gly Ser Trp Glu Val Leu Arg Phe Leu Leu Ser
 385 390 395 400
 Asn Ala Arg Trp Trp Leu Glu Glu Tyr Lys Phe Asp Gly Phe Arg Phe
 405 410 415
 Asp Gly Val Thr Ser Met Met Tyr Thr His His Gly Leu Gln Met Thr
 420 425 430
 Phe Thr Gly Asn Tyr Gly Glu Tyr Phe Gly Phe Ala Thr Asp Val Asp
 435 440 445
 Ala Val Val Tyr Leu Met Leu Val Asn Asp Leu Ile His Gly Leu His
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 Pro Asp Ala Val Ser Ile Gly Glu Asp Val Ser Gly Met Pro Thr Phe
 465 470 475 480
 Cys Ile Pro Val Pro Asp Gly Gly Val Gly Phe Asp Tyr Arg Leu His
 485 490 495
 Met Ala Val Ala Asp Lys Trp Ile Glu Leu Leu Lys Gln Ser Asp Glu
 500 505 510
 Ser Trp Lys Met Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp
 515 520 525
 Leu Glu Lys Cys Val Thr Tyr Ala Glu Ser His Asp Gln Ala Leu Val
 530 535 540
 Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp
 545 550 555 560
 Phe Met Ala Leu Asp Arg Pro Ser Thr Pro Arg Ile Asp Arg Gly Ile
 565 570 575
 Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly Gly Glu
 580 585 590
 Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile
 595 600 605
 Asp Phe Pro Arg Gly Pro Gln Thr Leu Pro Thr Gly Lys Val Leu Pro
 610 615 620
 Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly
 625 630 635 640
 Asp Ala Asp Phe Leu Arg Tyr His Gly Met Gln Glu Phe Asp Gln Ala
 645 650 655
 Met Gln His Leu Glu Glu Lys Tyr Gly Phe Met Thr Ser Glu His Gln
 660 665 670
 Tyr Val Ser Arg Lys His Glu Glu Asp Lys Val Ile Ile Phe Glu Arg
 675 680 685
 Gly Asp Leu Val Phe Val Phe Asn Phe His Trp Ser Asn Ser Phe Phe
 690 695 700
 Asp Tyr Arg Val Gly Cys Ser Arg Pro Gly Lys Tyr Lys Val Ala Leu
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 Asp Ser Asp Asp Ala Leu Phe Gly Gly Phe Ser Arg Leu Asp His Asp
 725 730 735
 Val Asp Tyr Phe Thr Thr Glu His Pro His Asp Asn Arg Pro Arg Ser
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 Phe Ser Val Tyr Thr Pro Ser Arg Thr Ala Val Val Tyr Ala Leu Thr
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<210> 9
 <211> 2511
 <212> DNA
 <213> Triticum aestivum

<400> 9

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<210> 10

<211> 836

<212> PRT

<213> *Triticum aestivum*

<400> 10

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          35          40          45
Pro Arg Ala Val Gly Val Gly Gly Ser Gly Trp Arg Val Val Met Arg
          50          55          60
Ala Gly Gly Pro Ser Gly Glu Val Met Ile Pro Asp Gly Gly Ser Gly
65          70          75          80
Gly Thr Pro Pro Ser Ile Asp Gly Pro Val Gln Phe Asp Ser Asp Asp
          85          90          95

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Glu	Asp	Ser	Ile	Trp	Ser	Ser	Glu	Thr	Asn	Gln	Val	Ser	Glu	Glu	Ile	115	120	125
Asp	Ala	Glu	Asp	Thr	Ser	Arg	Met	Asp	Lys	Glu	Ser	Ser	Thr	Arg	Glu	130	135	140
Lys	Leu	Arg	Ile	Leu	Pro	Pro	Pro	Gly	Asn	Gly	Gln	Gln	Ile	Tyr	Glu	145	150	155
Ile	Asp	Pro	Thr	Leu	Arg	Asp	Phe	Lys	Tyr	His	Leu	Glu	Tyr	Arg	Tyr	165	170	175
Ser	Leu	Tyr	Arg	Arg	Ile	Arg	Ser	Asp	Ile	Asp	Glu	His	Glu	Gly	Gly	180	185	190
Met	Asp	Val	Phe	Ser	Arg	Gly	Tyr	Glu	Lys	Phe	Gly	Phe	Met	Arg	Ser	195	200	205
Ala	Glu	Gly	Ile	Thr	Tyr	Arg	Glu	Trp	Ala	Pro	Gly	Ala	Asp	Ser	Ala	210	215	220
Ala	Leu	Val	Gly	Asp	Phe	Asn	Asn	Trp	Asp	Pro	Asn	Ala	Asp	His	Met	225	230	235
Ser	Lys	Asn	Asp	Leu	Gly	Val	Trp	Glu	Ile	Phe	Leu	Pro	Asn	Asn	Ala	245	250	255
Asp	Gly	Ser	Pro	Pro	Ile	Pro	His	Gly	Ser	Arg	Val	Lys	Val	Arg	Met	260	265	270
Gly	Thr	Pro	Ser	Gly	Thr	Lys	Asp	Ser	Ile	Pro	Ala	Trp	Ile	Lys	Tyr	275	280	285
Ser	Val	Gln	Thr	Pro	Gly	Asp	Ile	Pro	Tyr	Asn	Gly	Ile	Tyr	Tyr	Asp	290	295	300
Pro	Pro	Glu	Glu	Glu	Lys	Tyr	Val	Phe	Lys	His	Pro	Gln	Pro	Lys	Arg	305	310	315
Pro	Lys	Ser	Leu	Arg	Ile	Tyr	Glu	Thr	His	Val	Gly	Met	Ser	Ser	Pro	325	330	335
Glu	Pro	Lys	Ile	Asn	Thr	Tyr	Ala	Asn	Phe	Arg	Asp	Glu	Val	Leu	Pro	340	345	350
Arg	Ile	Lys	Arg	Leu	Gly	Tyr	Asn	Ala	Val	Gln	Ile	Met	Ala	Ile	Gln	355	360	365
Glu	His	Ser	Tyr	Tyr	Gly	Ser	Phe	Gly	Tyr	His	Val	Thr	Asn	Phe	Phe	370	375	380
Ala	Pro	Ser	Ser	Arg	Phe	Gly	Ser	Pro	Glu	Asp	Leu	Lys	Ser	Leu	Ile	385	390	395
Asp	Arg	Ala	His	Glu	Leu	Gly	Leu	Val	Val	Leu	Met	Asp	Val	Val	His	405	410	415
Ser	His	Ala	Ser	Asn	Asn	Thr	Leu	Asp	Gly	Leu	Asn	Gly	Phe	Asp	Gly	420	425	430
Thr	Asp	Thr	His	Tyr	Phe	His	Gly	Gly	Ser	Arg	Gly	His	His	Trp	Met	435	440	445
Trp	Asp	Ser	Arg	Val	Phe	Asn	Tyr	Gly	Asn	Lys	Glu	Val	Ile	Arg	Phe	450	455	460
Leu	Leu	Ser	Asn	Ala	Arg	Trp	Trp	Leu	Glu	Glu	Tyr	Lys	Phe	Asp	Gly	465	470	475
Phe	Arg	Phe	Asp	Gly	Ala	Thr	Ser	Met	Met	Tyr	Thr	His	His	Gly	Leu	485	490	495
Gln	Val	Thr	Phe	Thr	Gly	Ser	Tyr	His	Glu	Tyr	Phe	Gly	Phe	Ala	Thr	500	505	510
Asp	Val	Asp	Ala	Val	Val	Tyr	Leu	Met	Leu	Met	Asn	Asp	Leu	Ile	His	515	520	525
Gly	Phe	Tyr	Pro	Glu	Ala	Val	Thr	Ile	Gly	Glu	Asp	Val	Ser	Gly	Met	530	535	540
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Arg	Leu	His	Met	Ala	Val	Ala	Arg	Lys	Trp	Ile	Glu	Leu	Leu	Lys	Gly	565	570	575
Asn	Asp	Glu	Ala	Trp	Glu	Met	Gly	Asn	Ile	Val	His	Thr	Leu	Thr	Asn	580	585	590
Arg	Arg	Trp	Leu	Glu	Lys	Cys	Val	Thr	Tyr	Ala	Glu	Ser	His	Asp	Gln	595	600	605

Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp
610 615 620
Met Tyr Asp Phe Met Ala Leu Asn Gly Pro Ser Thr Pro Asn Ile Asp
625 630 635 640
Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu Ile Thr Met Gly Leu
645 650 655
Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro
660 665 670
Glu Trp Ile Asp Phe Pro Arg Gly Pro Gln Val Leu Pro Ser Gly Lys
675 680 685
Phe Ile Pro Gly Asn Asn Asn Ser Tyr Asp Lys Cys Arg Arg Arg Phe
690 695 700
Asp Leu Gly Asp Ala Glu Phe Leu Arg Tyr His Gly Met Gln Gln Phe
705 710 715 720
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725 730 735
Asp His Gln Tyr Val Ser Arg Lys His Glu Glu Asp Lys Val Ile Val
740 745 750
Phe Glu Lys Gly Asp Leu Val Phe Val Phe Asn Phe His Trp Ser Ser
755 760 765
Ser Tyr Phe Asp Tyr Arg Val Gly Cys Leu Lys Pro Gly Lys Tyr Lys
770 775 780
Val Val Leu Asp Ser Asp Ala Gly Leu Phe Gly Gly Phe Gly Arg Ile
785 790 795 800
His His Thr Ala Glu His Phe Thr Ser Asp Cys Gln His Asp Asn Arg
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16

30725-968PPH

CLAIMS:

1. A maize plant cell comprising a heterologous alpha-glucan, water dikinase (GWD) coding sequence and an amylose extender mutation.
2. The maize plant cell of claim 1 which is present in a maize plant.
- 5 3. The maize plant cell of claim 2, wherein said maize plant synthesizes a maize starch that has an increased apparent amylose content relative to maize starch from a corresponding wild-type maize plant, and wherein said maize starch further displays a measurable pasting temperature in an aqueous suspension in RVA analysis, wherein the RVA analysis is measured according to the AACC standard
10 method 61-02.
4. The maize plant cell of claim 3, wherein the aqueous suspension is a 10% (w/v) aqueous suspension of the maize starch in water.
5. The maize plant cell of claim 3, wherein the maize starch has a
15 phosphate content in position C6 between 15 and 30 nmol C6P/mg starch (dry weight).
6. The maize plant cell of any one of claims 1 to 5, which is a cell of reproductive material.
7. The maize plant cell of any one of claims 1 to 6, wherein said GWD coding sequence comprises the coding region of the nucleic acid sequence of
20 SEQ ID NO: 1.
8. The maize plant cell of any one of claims 1 to 6, wherein said GWD coding sequence comprises the coding nucleotide sequence set forth in SEQ ID NO: 3.
9. The maize plant cell of any one of claims 1 to 8, wherein said amylose
25 extender mutation comprises a mutation in SEQ ID NO: 5.

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10. A method for the production of maize starch comprising extracting maize starch from a maize plant comprising the maize plant cell of any one of claims 1 to 9.

11. A method for the production of maize meal comprising grinding a maize
5 plant comprising the maize plant cell of any one of claims 1 to 9.

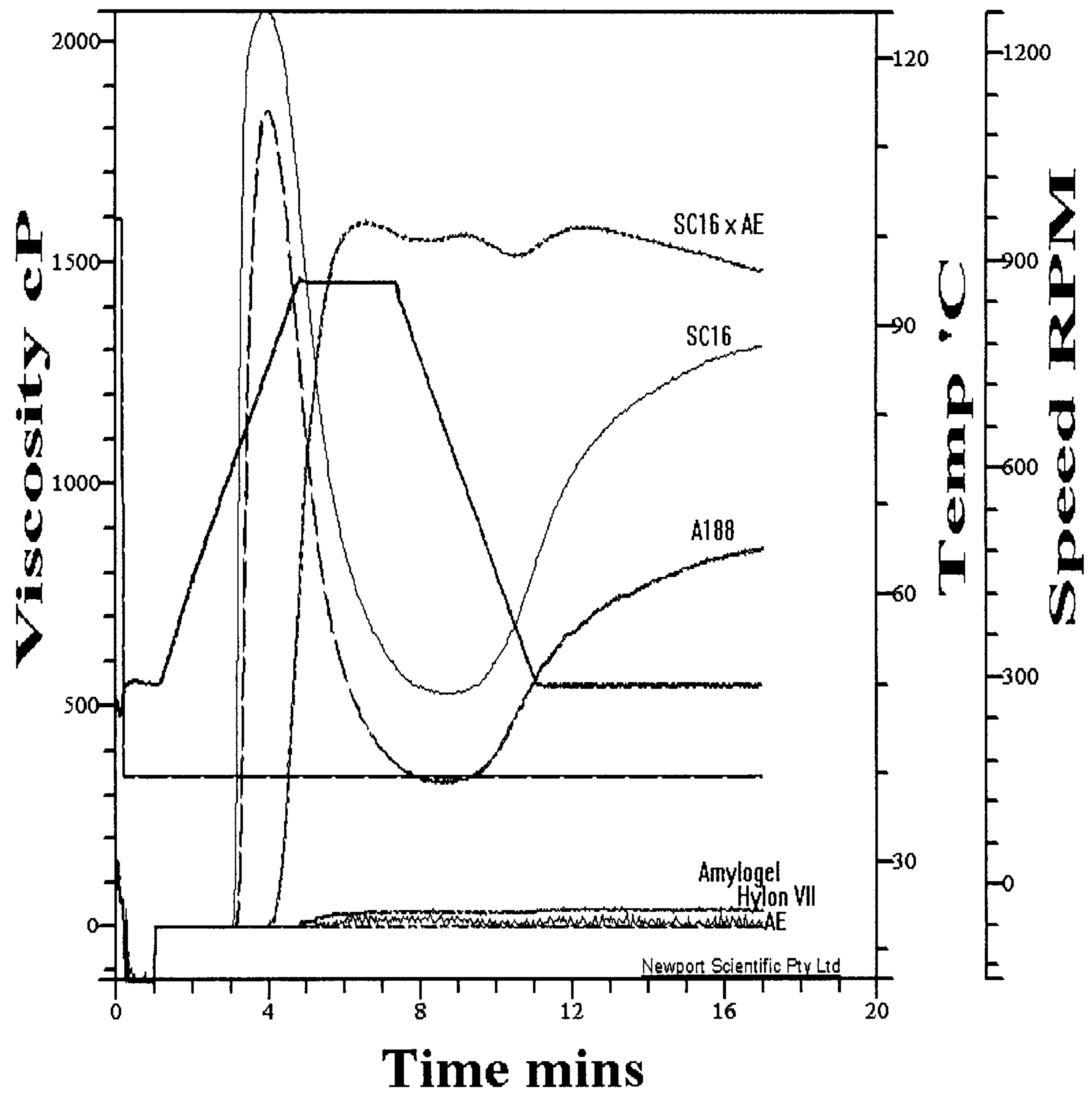
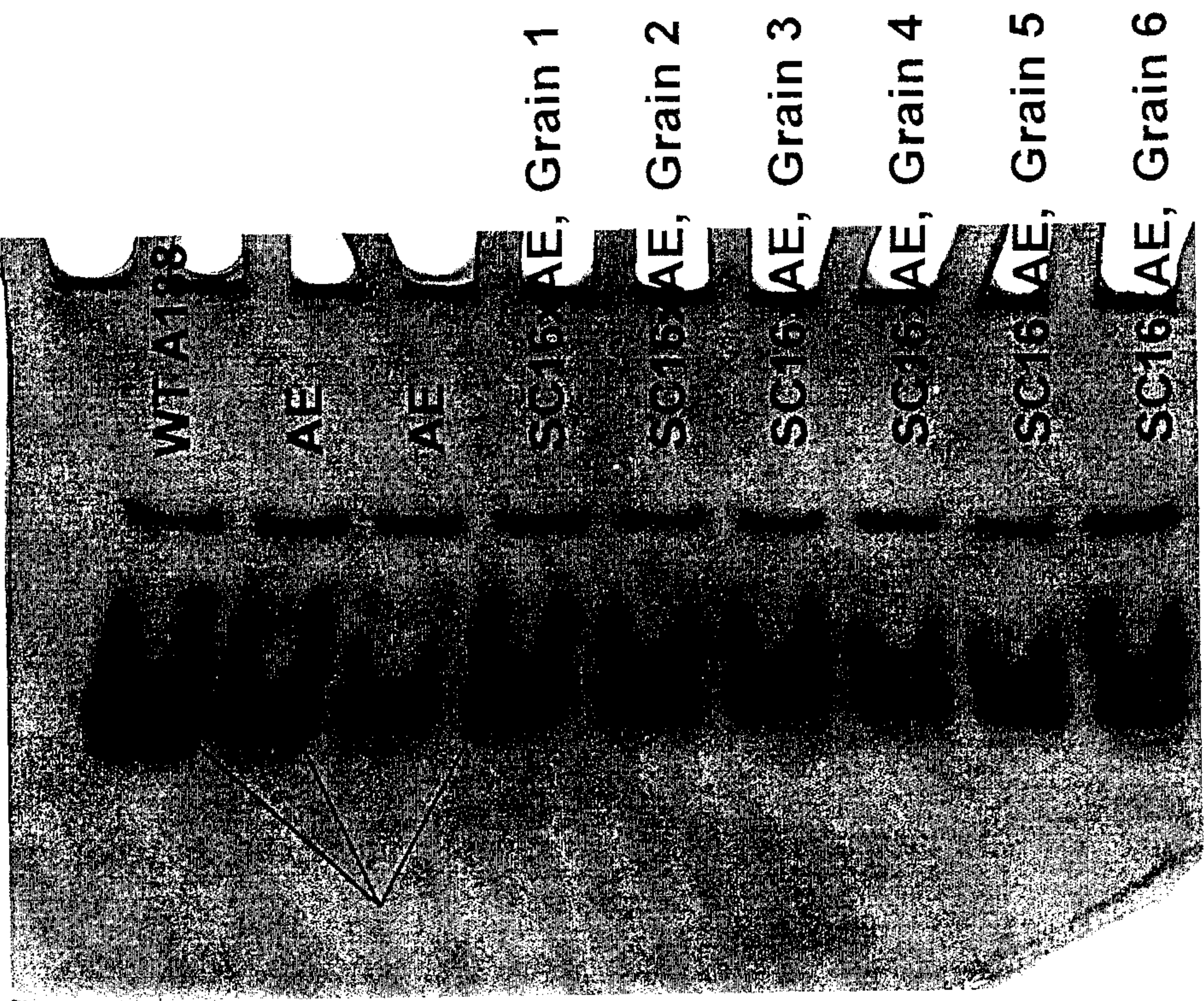


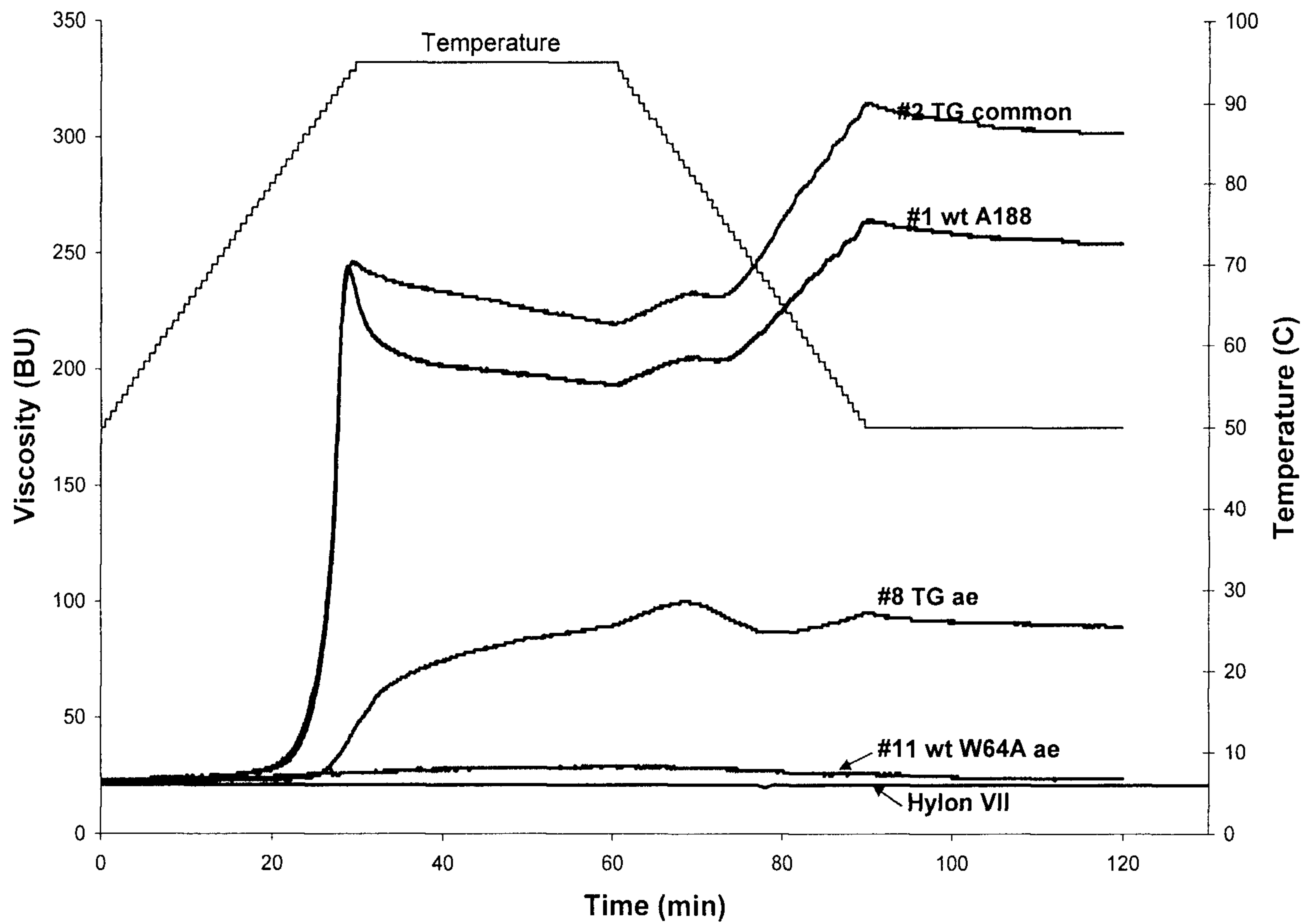
Figure 1



2 µg/ Slot

Figure 2

3/3



#1 = Maize-wildtype plants A188
#2 = SC16
#8 = SC 16 x ae1
#11 = ae1

Figure 3