



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 06, 2017

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *15/135,106*

FILING DATE: *April 21, 2016*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US15/135,106*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office



M. TARVER
Certifying Officer

Electronic Acknowledgement Receipt	
EFS ID:	25560956
Application Number:	15135106
International Application Number:	
Confirmation Number:	8300
Title of Invention:	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY
First Named Inventor/Applicant Name:	Hongxin JIANG
Customer Number:	81552
Filer:	Jonathan M. Cousin
Filer Authorized By:	
Attorney Docket Number:	INGR-036/01US
Receipt Date:	21-APR-2016
Filing Date:	
Time Stamp:	16:56:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 1600
RAM confirmation Number	3667
Deposit Account	501283
Authorized User	COOLEY LLP
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	INGR03601US_Transmittal_.pdf	246519 a890e5b743df2cf401df1d07d3d434dd468c51f1	no	1
Warnings:					
Information:					
2	Application Data Sheet	INGR03601US_ADS.pdf	1452174 dcfb9a7f6450a52a19c27ead2dd99fd7a859e6d5	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3		INGR03601US_Application.pdf	1347032 e1aaafda984d458b72ec2374c9cbb6abcfbc1f9c	yes	50
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	35	
	Claims		36	40	
	Abstract		41	41	
	Drawings-only black and white line drawings		42	50	
Warnings:					
Information:					
4	Sequence Listing (Text File)	INGR-036-00US_ST25.txt	11795	no	0
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	35318 1e3dd39f7424f1da1f20ba0e83d907b19d9c1e9a	no	2
Warnings:					
Information:					

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1.53(b))	Attorney Docket No.	INGR-036/01US
	First Inventor	JIANG, Hongxin
	Title	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY
	Express Mail Label No.	

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450
1. ☐ Fee Transmittal Form (PTO/SB/17 or equivalent)	ACCOMPANYING APPLICATION PARTS
2. ☐ Applicant asserts small entity status. See 37 CFR 1.27.	10. ☐ Assignment Papers (cover sheet & document(s)) Name of Assignee _____
3. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.	11. ☐ 37 CFR 3.73(c) Statement ☐ Power of Attorney (when there is an assignee)
4. ☒ Specification [Total Pages 41] Both the claims and abstract must start on a new page. (See MPEP § 608.01(a) for information on the preferred arrangement)	12. ☐ English Translation Document (if applicable)
5. ☒ Drawing(s). (35 U.S.C. 113) [Total Sheets 9]	13. ☐ Information Disclosure Statement (PTO/SB/08 or PTO-1449) ☐ Copies of citations attached
6. Inventor's Oath or Declaration [Total Sheets _____] (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))	14. ☐ Preliminary Amendment
a. ☐ Newly executed (original or copy)	15. ☐ Return Receipt Postcard (MPEP § 503) (Should be specifically itemized)
b. ☐ A copy from a prior application (37 CFR 1.63(d))	16. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
7. ☒ Application Data Sheet *See Note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent)	17. ☐ Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
8. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) ☐ Landscape Table on CD	18. ☐ Other:
9. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. – c. are required)	
a. ☒ Computer Readable Form (CRF)	
b. ☐ Specification Sequence Listing on:	
i. ☐ CD-ROM or CD-R (2 copies); or	
ii. ☐ Paper	
c. ☐ Statements verifying identity of above copies	

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 must be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☒ The address associated with Customer Number: 81552 OR ☐ Correspondence address below					
Name					
Address					
City	State	Zip Code			
Country	Telephone	Email			
Signature	/Jonathan M. Cousin/		Date	April 21, 2016	
Name (Print/Type)	Jonathan Cousin		Registration No. (Attorney/Agent)	66,418	

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application is a Continuation of U.S. Application No. 15/090,926, filed April 5, 2016, the disclosure of which is herein incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

10 The present invention relates to maize starch derived from plants and grains, compositions and methods for producing such starch.

BACKGROUND OF THE INVENTION

15 The existing waxy sugary-2 double-mutant maize starch shows very good freeze/thaw stability. However, the viscosity of the existing waxy sugary-2 maize starch was substantially lower than commercial waxy maize starch, such as AMIOCA™ starch. In addition, the pasting temperature of the existing waxy sugary-2 double-mutant maize starch is substantially lower than AMIOCA™ starch. Therefore, there remains a need in the art for producing maize starch with good freeze/thaw stability, increased viscosity, and/or higher pasting temperature.

20 In this invention, we developed new maize starch with higher viscosity and/or higher pasting temperature than commercial waxy maize starch, such as AMIOCA™ starch, by introduction of low activity of GBSSI to the existing waxy sugary-2 double-mutant maize.

SUMMARY OF THE INVENTION

25 The present invention provides starch with novel functionality which has not been found in nature. In some embodiments, the starch comprises an amylose content of from about 2 wt % to about 20 wt % using an amylose/amylopectin assay kit, e.g., Megazyme®. In some embodiments, the starch comprises an aqueous starch pasting temperature that is at least about 5% greater than the starch pasting temperature of a control starch as measured using a Rapid
30 Visco Analyzer (RVA, Newport Scientific, Sydney, Australia). In some embodiments, the starch

is derived from a waxy-sugary 2 double mutant corn plant comprising at least one *wx-Stonor* (*wxS*) allele in the endosperm. In some embodiments, the waxy-sugary 2 double mutant corn plant comprises a recessive *su2* mutant allele.

5 In some embodiments, the starch of the present invention is produced from plants which are not transgenic (i.e., non-GMO) for the genotype that produces the disclosed and claimed starch.

In some embodiments, the amylose content of the starch is from about 8 wt % to about 15 wt %.

10 In some embodiments, the aqueous pasting temperature is from about 5% to about 15 % greater than that of a control starch.

In some embodiments, the starch further comprises an enthalpy change of crystallinity. In some embodiments, the enthalpy change of crystallinity is at least about 30 % greater than an enthalpy change of crystallinity of a control starch as measured using a differential scanning calorimeter (DSC). In some embodiments, the enthalpy change of crystallinity is from about 30
15 % to about 200 % greater than an enthalpy change of crystallinity of a control starch as measured using a DSC.

In some embodiments, the starch further comprises a distribution of starch granule sizes, wherein the distribution of starch granule sizes comprises at least about 40 % fewer granules less than 5 microns compared to a proportion of granules less than 5 microns from a control starch as
20 observed using a scanning electron microscope.

In some embodiments, the starch has a granule which exhibits increased integrity compared to a starch derived from the control corn plant as observed using a scanning electron microscope.

25 In some embodiments, the starch is capable of withstanding at least three freeze-thaw cycles. In some embodiments, the starch is capable of withstanding at least three to at least five freeze-thaw cycles.

In some embodiments, a plant producing the starch of the present invention is generated by cross pollination of a first corn plant having the genotype of *wxwxsu2su2*, and a second corn plant having the genotype of *wxSwxSsu2su2*, or the genotype of *wxSwxsu2su2*. In some
30 embodiments, the endosperm of the waxy-sugary 2 corn plant has one dose, two doses, or three

doses of the *wxS* allele, and the endosperm has the genotype of *wxSwxwxsu2su2su2*, *wxSwxSwxsu2su2su2*, or *wxSwxSwxSsu2su2su2*.

5 The present invention also provides methods for producing the starch with an improved pasting profile. In some embodiments, the methods comprise introducing at least one *wxS* allele into the endosperm of a waxy corn plant.

10 The present invention also provides novel double-mutant plants. In some embodiments, the plants are corn plants. In some embodiments, the plants are homozygous recessive for the starch synthase IIa (*su2*) gene. In some embodiments, the plants are either homozygous or heterozygous for a mutated Granule-bound starch synthase I (GBSSI) gene. In some
embodiments, the mutated GBSSI gene has less GBSSI activity as the wild type GBSSI gene (*Wx*), but has higher GBSSI activity than the recessive, loss-of-function GBSSI gene (*wx*).

15 In some embodiments, the mutated GBSSI gene has about 8% to about 10% of GBSSI activity of the wild type GBSSI gene. In some embodiments, the mutated GBSSI gene is the *wx-Stonor* (*wxS*) allele. In some embodiments, the mutated GBSSI gene is the *wx-G* allele, the *wx-B5* allele, or the *wx-M* allele.

In some embodiments, the double-mutant plant is generated by cross pollination of two parent plants. In some embodiments, the first plant has the genotype of *wxwxsu2su2*. In some embodiments, the second plant has the genotype of *wxSwxSsu2su2*, or the genotype of *wxSwxsu2su2*.

20 In some embodiments, the endosperm of the plant has one dose, two doses, or three doses of the *wxS* allele. In some embodiments, the endosperm of the plant has the genotype of *wxSwxwxsu2su2su2*, *wxSwxSwxsu2su2su2*, or *wxSwxSwxSsu2su2su2*.

25 In some embodiments, starch produced in the endosperm of the plant has a higher viscosity compared to the starch produced in the endosperm of a double, waxy sugary-2 recessive mutant plant having the genotype of *wxwxwxsu2su2su2*.

In some embodiments, starch produced in the endosperm of the plant has a higher starch pasting temperature compared to the starch produced in the endosperm of a double, waxy sugary-2 recessive mutant plant having the genotype of *wxwxwxsu2su2su2*.

The present invention also provides plant parts, plant cells, and tissue cultures of the plants. In some embodiments, the plant part is an endosperm, a leaf, a flower, an ovule, a pollen, a rootstock, or a scion. In some embodiments, the plant part is a seed of the plants.

The present invention also provides starch extracted from plants described above.

5 The present invention also provides methods for producing starch. In some embodiments, the methods comprise obtaining starch from the endosperm of the plants of the present invention.

In some embodiments, the methods for producing starch comprise growing the plants of the present invention, and harvesting seeds from the plants.

10 The present invention also provides methods for producing a seed. In some embodiments, the methods comprise crossing a first parent plant with a second parent plant and harvesting the resultant seed. In some embodiments, said first parent plant and/or second parent plant is a plant of the present invention. In some embodiments, the methods comprise self-pollinating a plant of the present invention, and harvesting the resultant seed.

15 The present invention also provides methods of vegetatively propagating the plant. In some embodiments, the methods comprise collecting part of the plant and regenerating a plant from said part.

The present invention also provides methods of producing a plant derived from the plants of the present invention. In some embodiments, the methods comprise self-pollinating a plant of the present invention at least once to produce a progeny plant derived from the plant. In some
20 embodiments, the methods comprise crossing a plant of the present invention with a second plant to produce a plant progeny.

The present invention also provides methods of producing a transgenic plant. In some embodiments, the methods comprise introducing a transgene of interest into a plant of the present invention. The transgene of interest confers one or more desired phenotypes in the plant.
25

The present invention also provides methods of producing a plant with desired phenotypes, comprising gene-editing to produce a genetic sequence in a plant of the present invention. The genetic sequence confers one or more desired phenotypes in the plant.

The present invention also provides methods of producing a plant with desired
30 phenotypes, comprising inducing one or more mutations into a plant of the present invention to

produce a genetic sequence. The genetic sequence confers one or more desired phenotypes in the plant.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the pasting profiles of AMIOCA™ starch, *wxwxwxSU2su2su2* starch, and starch of *waxy-sugary 2* double-mutant plants with zero, one, two, or three doses of *wxS* gene, as measured by a Rapid Visco Analyzer (RVA) over time during the heating-cooling cycle.

10 FIG. 2 depicts the pasting properties of AMIOCA™ starch, *wxwxwxSU2su2su2* starch, and starch of *waxy-sugary 2* double-mutant plants with zero, one, two, or three doses of *wxS* gene, including the pasting temperature, peak RVU, hold RVU, final RVU, breakdown RVU, and setback RVU.

15 FIG. 3 depicts the freeze-thaw stability test of starch of *waxy-sugary 2* double-mutant plants with one, two, three, or zero doses of *wxS* gene, starch from Waxy Stonor seed, *wxwxwxSU2su2su2* starch, and AMIOCA™ starch. The above-stated starches are presented in rows, and illustrative images of starch gels are provided after the first freeze-thaw (Cycle 1), after the third freeze-thaw (Cycle 3), and after the fifth freeze-thaw (Cycle 5).

20 FIG. 4 depicts the thermal properties of AMIOCA™ starch, *wxwxwxSU2su2su2* starch, and starch of *waxy-sugary 2* double-mutant plants with zero, one, two, or three doses of *wxS* gene.

FIG. 5 depicts the granule size distribution of starch of AMIOCA™ starch, *wxwxwxSU2su2su2* starch, and starch of *waxy-sugary 2* double-mutant plants with zero, one, two, or three doses of *wxS* gene.

25 FIG. 6 depicts the granule morphology of starch of *waxy-sugary 2* double-mutant plants with one, two, three, or zero doses of *wxS* gene, *wxwxwxSU2su2su2* starch, AMIOCA™ starch, and starch from Waxy Stonor seed. Illustrative images for each of the above-referenced starches captured using a scanning electron microscope are provided.

30

DETAILED DESCRIPTION OF THE INVENTION***Definitions***

As used herein, the verb "comprise" as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are
5 included, but items not specifically mentioned are not excluded.

The invention provides compositions and methods to produce plants. As used herein, the term "plant" refers to any living organism Plantae (i.e., any genus/species in the Plant Kingdom). In some embodiments, the plant is capable of producing starch, including but not limited to maize, potato, wheat, tapioca, rice, potato and sorghum. In some embodiments, the plant is a
10 corn plant.

The invention provides plant parts. As used herein, the term "plant part" refers to any part of a plant including but not limited to the embryo, endosperm, shoot, root, stem, seed, stipule, leaf, petal, flower, ovule, bract, branch, petiole, internode, bark, pubescence, tiller, rhizome, frond, blade, ovule, pollen, stamen, and the like.

15 The invention provides genes comprising the isolated (e.g., wild type, endogenous to the organism), chimeric, recombinant or synthetic nucleic acid sequence. As used herein, the term "gene" refers to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include nonexpressed DNA segments that, for example, form
20 recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

The invention provides polynucleotides with nucleotide change when compared to a wild-type reference sequence. As used herein, the term "nucleotide change" refers to, e.g.,
25 nucleotide substitution, deletion, insertion, or transposon, as is well understood in the art. For example, mutations contain alterations that produce silent substitutions, additions, deletions, or alternative splicing, which either alter or do not alter the properties or activities of the encoded protein or how the proteins are made. A nucleotide change can also be insertion and/or exclusion of a retrotransposon element in the gene.

The invention provides polypeptides with protein modification when compared to a wild-type reference sequence. As used herein, the term “protein modification” refers to, e.g., amino acid substitution, amino acid modification, deletion, insertion, or change of protein activity, as is well understood in the art.

5 The invention provides polynucleotides and polypeptides derived from wild-type reference sequences. As used herein, the term “derived from” refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid or an amino acid derived from an origin or source may have all kinds of nucleotide changes or protein modification as defined elsewhere herein.

10 The invention provides biologically active variants or functional variants of the nucleic acid sequences and polypeptide sequences of the present invention. As used herein, the phrase “a biologically active variant” or “functional variant” with respect to a protein refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence, while still maintains substantial biological activity of the reference sequence. The variant can
15 have “conservative” changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. The following table shows exemplary conservative amino acid substitutions. In some embodiments, the variant has one or more amino acid substitutions, wherein one or more or all substitutions are acidic amino acid, such as Aspartic acid, Asparagine, Glutamic acid, or Glutamine.

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr

Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val
Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

Alternatively, a variant can have “nonconservative” changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations can also include amino acid deletion or insertion, or both. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without eliminating biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR software. For polynucleotides, a variant comprises a polynucleotide having deletions (i.e., truncations) at the 5' and/or 3' end; deletion and/or addition of one or more nucleotides at one or more internal sites in the reference polynucleotide; and/or substitution of one or more nucleotides at one or more sites in the reference polynucleotide. As used herein, a “reference” polynucleotide comprises a nucleotide sequence produced by the methods disclosed herein. Variant polynucleotides also include synthetically derived polynucleotides, such as those generated, for example, by using site directed mutagenesis but which still comprise genetic regulatory element activity. Generally, variants of a particular polynucleotide or nucleic acid molecule, or polypeptide of the invention will have at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 91.5%, 92%, 92.5%, 93%, 93.5%, 94%, 94.5%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more sequence identity to that particular polynucleotide/polypeptides as determined by sequence alignment programs and parameters as described elsewhere herein.

Variant polynucleotides also encompass sequences derived from a mutagenic and recombinogenic procedure such as DNA shuffling. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *PNAS* 91:10747-10751; Stemmer (1994) *Nature*

370:389-391; Cramer *et al.* (1997) *Nature Biotech.* 15:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347; Zhang *et al.* (1997) *PNAS* 94:4504-4509; Cramer *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458. For PCR amplifications of the polynucleotides disclosed herein, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al.*, eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

The invention provides transgenic plants. As used herein, the term "transgenic" refers to cells, cell cultures, organisms (e.g., plants), and progeny which have received a foreign or modified gene by one of the various methods of transformation, wherein the foreign or modified gene is from the same or different species than the species of the organism receiving the foreign or modified gene.

Waxy gene: Waxy gene is the granule-bound starch synthase I (GBSSI) gene. The gene encodes a starch granule-bound ADP-glucose-glucosyl transferase (EC 2.4.1.242) responsible for amylose biosynthesis in the triploid endosperm of the developing kernel and in haploid pollen and embryo sac. "Wx" refers to the wild type allele, and "wx" refers to the null, reference mutant-allele. The terms *wxS* (a.k.a. *wx-Stonor*), *wx-G*, *wx-B5*, and *wx-M* refer to the intermediate alleles having retrotransposon insertion in the waxy gene, as described in Wessler and Varagona (*PNAS*, 82:4177-4181, 1985) and Varagona *et al.* (*The Plant Cell*, 4:811-820, 1992), each of which is herein incorporated by reference in its entirety. These intermediate alleles result in less waxy protein activity in plants having the alleles than the waxy protein activity in wild type plants. The maize waxy gene encodes the protein having the sequence of SEQ ID NO: 1.

5 *wx-Stonor*: The *wx-Stonor* allele in maize plant is caused by a retrotransposon insertion at the splice donor site of intron 5 of the waxy gene. Although the insertion does not affect the splicing machinery, it leads to a lower level of enzymatic activity compared to the wild type allele (e.g., only about 9.5% GBSSI activity as of the wild type). As a result, plants having *wx-Stonor* allele has higher amylose level than the same plant which does not have the *wx-Stonor* allele.

10 Sugary-2 gene: Sugary-2 gene encodes starch synthase isoform IIa (SSIIa, EC 2.4.1.21). “*SU2*” refers to the wild type allele, and “*su2*” refers to the recessive, mutant allele. The function of Sugary-2 protein in maize is described in Zhang et al. (Plant Molecular Biology, 54(6):865-879, 2004), which is herein incorporated by reference in its entirety. The maize wild type *SU2* gene encodes the wild type protein having the sequence of SEQ ID NO: 2.

More detail of the waxy gene and the sugary-2 gene in maize are described in U.S. Patent Nos. 4428972, 5954883, 6828474, 6960703, and 7678898, each of which is herein incorporated by reference in its entirety.

15 AMIOCA™ starch: the term refers to the AMIOCA™ corn starch produced by National Starch and Chemical Company, now Ingredion Incorporated.

wxwxwxSU2su2su2 starch: the term refers to starch isolated from maize endosperm having the genotype of *wxwxwxSU2su2su2*.

20 *wxwxwxsu2su2su2* starch: the term refers to starch isolated from maize endosperm having the genotype of *wxwxwxsu2su2su2*, which is the endosperm of the waxy sugary-2 double recessive mutant. The mutated waxy gene contributes to its good freeze-thaw stability, and the mutated sugary-2 gene results in short branch-chain length. However, the starch has low integrity of starch granules. The granules break down easily, and the starch has low viscosity.

25 MELOJEL® starch: the term refers to the MELOJEL® corn starch produced by National Starch and Chemical Company, now Ingredion Incorporated.

Control corn plant: the term refers to a proper check plant for comparison. In some embodiments, a check plant can be a wild type plant. In some embodiments, the check plant is a plant that does not have an intermediate waxy gene as described herein. In some embodiments, the check plant is a corn plant that produces a *wxwxwxSU2su2su2* starch or a *wxwxwxsu2su2su2*

starch. Accordingly, in some embodiments, the control corn plant is a corn plant which does not possess a *wx-S* allele in the endosperm.

Control starch: the term refers to a control starch.

wxSwxwxsu2su2su2: the term refers to a maize endosperm with one dose of *wxS*.

5 *wxSwxwxsu2su2su2* starch: the term refers to a starch isolated from maize endosperm with one dose of *wxS*, the endosperm having the genotype *wxSwxwxsu2su2su2*.

wxSwxSwxsu2su2su2: the term refers to a maize endosperm with two doses of *wxS*.

wxSwxSwxsu2su2su2 starch: the term refers to a starch isolated from maize endosperm with two doses of *wxS*, the endosperm having the genotype *wxSwxSwxsu2su2su2*.

10 *wxSwxSwxSsu2su2su2*: the term refers to a maize endosperm with three doses of *wxS*.

wxSwxSwxSsu2su2su2 starch: the term refers to a starch isolated from maize endosperm with three doses of *wxS*, the endosperm having the genotype *wxSwxSwxSsu2su2su2*.

15 The following examples illustrate various aspects of the invention. The examples should, of course, be understood to be merely illustrative of only certain embodiments of the invention and not to constitute limitations upon the scope of the invention.

Starch with Novel Functionalities

In one aspect, the disclosure provides for a starch derived from a double-mutant corn plant comprising an intermediate GBSSI allele which has less GBSSI activity than a wild type
20 GBSSI gene (*Wx*), but has higher GBSSI activity than a recessive, loss-of-function GBSSI gene (*wx*). In some embodiments, the double-mutant corn plant with an intermediate GBSSI allele is a waxy-sugary 2 corn plant comprising at least one *wx-Stonor* (*wxS*) allele in the endosperm. In some embodiments, the waxy-sugary 2 corn plant is generated by cross pollination of a first corn plant having the genotype of *wxwxsu2su2*, and a second corn plant having the genotype of
25 *wxSwxSsu2su2* or the genotype of *wxSwxsu2su2*. In further embodiments, the endosperm of the waxy-sugary 2 corn plant has one dose, two doses, or three doses of the *wxS* allele, and the endosperm has the genotype of *wxSwxwxsu2su2su2*, *wxSwxSwxsu2su2su2*, or *wxSwxSwxSsu2su2su2*.

30 In some embodiments, the starch comprises an amylose content of from about 2 wt % to about 20 wt % as measured using a Megazyme® amylose/amylopectin assay kit, and an aqueous

starch pasting temperature that is at least about 5% greater than the pasting temperature of a control starch as measured using RVA analysis.

Amylose Content. Starch is comprised of amylose and amylopectin. Amylose is a helical polymer made of α -D-glucose units, connected through $\alpha(1\rightarrow4)$ glycosidic bonds. Amylopectin is a highly branched polymer of glucose comprising $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glycosidic bonds. Branching takes place with the $\alpha(1\rightarrow6)$ bonds, which occurs at approximately every 24 to 30 glucose units. In some embodiments, the amylose content in starch can be measured using an amylose/amylopectin assay kit, such as the amylose/amylopectin assay kit manufactured by Megazyme®, as set forth in the Examples section.

An intermediate mutation of the GBSSI gene in the double-mutant corn plant was observed to increase amylose content in the starch derived therefrom relative to a control corn plant with the null-allele GBSSI gene. For example, amylose content of a starch derived from a waxy-sugary 2 corn plant comprising at least one *wx-Stonor* (*wxS*) allele in the endosperm was observed to increase relative to a control corn plant. In some embodiments, the starch derived from a corn plant with at least one *wx-Stonor* (*wxS*) allele in the endosperm has an amylose content of from about 2 wt % to about 20 wt %, from about 4 wt % to about 18 wt %, from about 5 wt % to about 15 wt %, or about 8 wt % to about 15 wt %, including all values and subranges in between. In other embodiments, the amylose content is about 5 wt %, about 6 wt %, about 7 wt %, about 8 wt %, about 9 wt %, about 10 wt %, about 11 wt %, about 12 wt %, about 13 wt %, about 14 wt %, about 15 wt %, about 16 wt %, about 17 wt %, or about 18 wt %, including all values between.

Without being limited by theory, an increase in the amylose content would be expected to decrease the amylopectin content in the starch, thereby decreasing the total number glucose units and hydrogen-bonding groups available to interact with an aqueous medium. Such a decrease in hydrogen bonding should correspond to an overall decrease in the strength of the interaction of said starch with the aqueous medium and diminish the pasting properties of the starch. However, an unexpected improvement in the pasting profile was observed when the amylose content was increased in the starch disclosed herein. Without being limited by theory, this unexpected result can be attributed to an increase in the branch-chain length observed in a waxy-sugary 2 corn plant comprising at least one *wx-Stonor* (*wxS*) allele in the endosperm. Longer branch chains on

the starch present more glucose units, which increase the number of hydrogen bonding interactions with the aqueous medium compared to shorter branch chain lengths.

The pasting profile of a starch can be measured by known methods, such as using a Rapid Visco Analyzer (RVA) (Newport Scientific, Sydney, Australia). For example, RVA can measure pasting temperature (°C), peak viscosity (RVU), hold viscosity (RVU), final viscosity (RVU), breakdown viscosity (RVU), and set back viscosity (RVU). In some embodiments, the improved pasting profile allows gelatinization to occur, *inter alia*, at a lower effective amount of the starch and/or at a higher pasting temperature compared to a control starch. Starch gelatinization refers to a process of breaking intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (e.g., hydroxyl groups) to bind more water. To measure a starch pasting profile, an effective amount of a starch can be added to an aqueous solution at neutral pH. For example, starch can be added to an aqueous solution at 8 w/w % solids level.

Pasting Temperature. Pasting temperature, as used herein, is intended to be measured as set forth in the Examples section. The pasting temperature of starch is the temperature at which initial swelling of starch granules takes place when suspended in water. In some embodiments, the starch described herein has an aqueous pasting temperature that is at least about 5% greater than the pasting temperature of a control starch as measured using RVA analysis. In some embodiments, the aqueous pasting temperature is increased by about 1% to about 100 %, about 1% to about 50 %, about 5% to about 50%, about 5% to 40%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20% or about 5% to about 14 %, including all values and subranges in between. In a particular embodiment, the aqueous pasting temperature is from about 5% to about 14 % greater than that of a control corn plant, such as a double mutant waxy-sugary 2 corn plant.

In other embodiments, the aqueous pasting temperature is increased by at least about 2%, at least about 3%, at least about 4%, at least about 5 %, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, or at least about 20% greater compared to a pasting temperature of a control starch, including all values in between.

In still other embodiments, the aqueous pasting temperature is increased by 1 °C to about 20 °C, or about 5 °C to about 10 °C, including all values and subranges in between. In other embodiments, the aqueous pasting temperature is increased at least about 4 °C, at least about 5 °C, at least about 6 °C, at least about 7 °C, at least about 8 °C, at least about 9 °C, at least about 10 °C, at least about 11 °C, at least about 12 °C, at least about 13 °C, at least about 14 °C, or at
5 or about 15 °C compared to a pasting temperature of a control starch.

In still other embodiments, the starch described herein has an aqueous pasting temperature of at least about 50 °C, at least about 55 °C, at least about 60 °C, at least about 65 °C, at least about 70 °C, at least about 75 °C, at least about 80 °C, at least about 85 °C, at least about
10 90 °C, at least about 95 °C, or at least about 100 °C. In particular embodiments, the starch derived from a waxy-sugary 2 corn plant comprising at least one *wx-Stonor* (*wxS*) allele in the endosperm has an aqueous pasting temperature of from about 55 °C to about 68 °C, or from about 59 °C to about 65 °C, including all values and subranges in between. In yet other embodiments, the aqueous pasting temperature is about 59 °C, about 60 °C, about 61 °C, about
15 62 °C, about 63 °C, about 64 °C, about 65 °C, about 66 °C, about 67 °C, or about 68 °C, including all values in between.

Enthalpy Change of Crystallinity. Enthalpy change of crystallinity, as used herein, is intended to be measured as set forth in the Examples section. Crystallization refers to the process of forming solid crystals from a solution by precipitation. In some embodiments, introduction of
20 at least one *wx-Stonor* (*wxS*) allele in the endosperm of a in a waxy-sugary 2 double-mutant corn plant was observed to increase the enthalpy change of crystallinity relative to a starch from a control corn plant. Enthalpy change can be measured using, e.g., differential scanning calorimetry (DSC). In some embodiments, the enthalpy change of crystallinity is increased by about 10% to about 300%, about 10% to about 200%, about 10% to about 150%, about 10% to
25 about 100%, or about 10% to about 50%, including all values and subranges in between. In one such embodiment, the enthalpy change of crystallinity is from about 70 % to about 200 % greater than an enthalpy change of crystallinity of a control starch as measured using a DSC.

In some embodiments, the enthalpy change of crystallinity is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least
30 about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at

least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200%, including all values in between.

5 In some embodiments, the enthalpy change of crystallinity is from about 1 J/g to about 20 J/g, about 5 J/g to about 15 J/g, about 6 J/g to about 13 J/g, including all values and subranges in between. In other embodiments, the enthalpy change of crystallinity 8 w/w % is about 4 J/g, about 5 J/g, about 6 J/g, about 7 J/g, about 8 J/g, about 9 J/g, about 10 J/g, about 11 J/g, about 12 J/g, about 13 J/g, including all values in between. In a particular embodiment, the corn plant comprises one *wxS* allele in the endosperm, and the enthalpy change of crystallinity is at least
10 about 150% greater than that of a starch derived from a control plant with the endosperm genotype of *wxwxwxsu2su2su2*. In another particular embodiment, the corn plant comprises two *wxS* alleles in the endosperm, and the enthalpy change of crystallinity is at least about 30 % greater than that of a starch derived from a control plant with the endosperm genotype of *wxwxwxsu2su2su2*. In yet another particular embodiment, the corn plant comprises three *wxS*
15 alleles in the endosperm, and the enthalpy change of crystallinity is at least about 75 % greater than that of a starch derived from a control plant with the endosperm genotype of *wxwxwxsu2su2su2*.

Freeze Thaw Stability. Freeze thaw stability, as used herein, is intended to be measured as set forth in the Examples section. In some embodiments, the starch described herein exhibits
20 desirable freeze-thaw characteristics, e.g., improved freeze-thaw stability. Freeze-thaw stability can be measured by adding an effective amount of a starch to water to form a slurry, which is then cooked and syneresis is measured after cycles of freezing/cooling. In some embodiments, introduction of at least one *wx-Stonor* (*wxS*) allele into the endosperm of a corn plant did not reduce the freeze-thaw stability of the starch derived therefrom compared to a control starch. In
25 other embodiments, introduction of at least one *wx-Stonor* (*wxS*) allele into the endosperm of a corn plant improved the freeze-thaw stability of the starch. In one such embodiment, the starch is capable withstanding a total of at least three to at least five freeze-thaw cycles.

Granule Distribution and Integrity. Granule distribution and integrity, as used herein, is intended to be measured as set forth in the Examples section.

In some embodiments, introduction of at least one *wx-Stonor* (*wxs*) allele in the endosperm of a corn plant was observed to alter the distribution of sizes of starch granules relative to a control starch. In some such embodiments, the distribution of starch granule sizes of a starch described herein comprises at least about 40 % fewer granules less than 5 microns compared to a proportion of granules less than 5 microns from a control starch as observed using a scanning electron microscope.

Granule integrity, as used herein, refers to the ability of the starch to remain as an intact granule after dissolution in an aqueous medium. In some embodiments, the starch described herein is a granule which exhibits increased integrity compared to a starch derived from the control corn plant as observed using a scanning electron microscope.

Viscosity. Viscosity, as used herein, is intended to be measured as set forth in the Examples section. In some embodiments, the starch described herein has an aqueous peak viscosity that is at least about 10% greater than the aqueous peak viscosity of a control starch as measured using RVA analysis. In some embodiments, the aqueous peak viscosity was increased by about 10% to about 300%, about 10% to about 250%, about 10% to about 200%, about 10% to about 150%, about 10% to about 100%, or about 10% to about 50%, including all values and subranges in between. In some embodiments, the aqueous peak viscosity was increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120 %, at least about 130 %, at least about 140 %, at least about 150 %, at least about 160 %, at least about 170 %, at least about 180 %, at least about 190 %, or at least about 200 %, including all values in between.

In some embodiments, the aqueous peak viscosity of the starch at 8 w/w % solids is about 100 RVU to about 500 RVU, about 100 RVU to about 400 RVU, about 100 RVU to about 300 RVU, about 100 RVU to about 200 RVU, or about 100 RVU to about 150 RVU, including all values and subranges in between. In some embodiments, the aqueous peak viscosity is about 150 RVU, about 155 RVU, about 160 RVU, about 165 RVU, about 170 RVU, about 175 RVU, about 180 RVU, about 185 RVU, about 190 RVU, about 195 RVU, about 200 RVU, about 205 RVU, about 210 RVU, about 215 RVU, about 220 RVU, about 225 RVU, about 230 RVU, about 235 RVU, about 240 RVU, about 245 RVU, about 250 RVU, about 255 RVU, about 260 RVU, about

Extraction of flour or variants thereof from the plant can be accomplished by a dry-milling process. In a typical procedure which is suitable herein but not exclusive of other procedures, the grain obtained from a plant of the present invention is first thoroughly cleaned and passed through a scourer and is then tempered or conditioned and passed through a degerminator. Stock from the degerminator is dried and then cooled, passed through a hominy separator and aspirator, ground, and finally sifted according to whether whole or separate fractions are desired.

Modification. It can be understood that some modifications in either of the above extraction processes such, for example, as using a steep-water temperature below that which is commonly employed, may be desirable and will be easily recognized by a starch practitioner.

The starch herein may be modified, if desired, by procedures known in the art, such as by derivatization to form ethers or esters such as hydroxypropyl ethers, acetates, phosphates, succinates, e.g., octenyl succinate, tertiary and quaternary amine ethers, etc., or by any other modification techniques which produce a starch having the characteristics defined herein. The preferred substituent groups herein are hydroxypropyl, phosphate or acetate groups.

For commercial purposes, one modification of the starch herein is crosslinking to strengthen the granules against the handling and processing conditions frequently encountered in manufacturing operations and to provide a starch capable of imparting desirable rheological properties to food systems. Any cross-linking agent known in the art may be employed for this purpose, including but not limited to epichlorohydrin, linear dicarboxylic acid anhydrides, citric acid acrolein, phosphorus oxychloride, adipic/acetic mixed acid anhydrides, and trimetaphosphate salts for food systems and to epichlorohydrin, linear dicarboxylic acid anhydrides, citric acid acrolein, phosphorus oxychloride, adipic/acetic mixed acid anhydrides, trimetaphosphate salts, formaldehyde, cyanuric chloride, diisocyanates, and divinyl sulfones in non-food systems. The cross-linking reaction is carried out using techniques known in the art, for example those described in U.S. Pat. Nos. 2,328,537 and 2,801,242. Procedures for modifying starches are described in the Chapter "Starch and Its Modification" by M. W. Rutenberg, pages 22-26 to 22-47, Handbook of Water Soluble Gums and Resins, R. L. Davidson, Editor (McGraw-Hill, Inc., New York, N.Y. 1980).

The amount of crosslinking agent necessary to give a suitable product will vary depending, for example, on the type of crosslinking agent employed, the concentration of the crosslinking agent, the reaction conditions, and the necessity for having a crosslinked starch which falls within the desired viscosity range. The practitioner will recognize which amounts may be employed, as these are well known in the art. Typically, this amount will range from as low as about 0.001%, by weight of the starch, to as high as is considered acceptable for food use.

The present starches may also be physically modified, such as by thermal inhibition described in WO 95/04082 (published Feb. 9, 1995).

Processing. The starches may also be pregelatinized. Exemplary processes for preparing pregelatinized starches are disclosed in U.S. Pat. No. 4,280,851 (Pitchon, et al.), U.S. Pat. No. 4,465,702 (Eastman, et al.), U.S. Pat. No. 5,037,929 (Rajagopalan), U.S. Pat. No. 5,131,953 (Kasica, et al.), and U.S. Pat. No. 5,149,799 (Rubens). Conventional procedures for pregelatinizing starch are well known to those skilled in the art and described in such articles as Chapter XXII--"Production and Use of Pregelatinized Starch", Starch: Chemistry and Technology, Vol. III--Industrial Aspects, R. L. Whistler and E. F. Paschall, Editors, Academic Press, New York 1967.

The present starches may be purified by any method known in the art to remove off-flavors and colors that are native to the starch or created during starch modification processes. Purification processes preferred for treating the present starches are disclosed in U.S. Ser. No. 07/832,838 filed Feb. 7, 1992, by Kasica, et al. Alkali washing techniques, for starches intended for use in either granular or pregelatinized form, are also useful and described in the family of patents represented by U.S. Pat. No. 5,187,272 (Bertalan, et al.).

Conversion products derived from the present starches, including but not limited to fluidity or thin-boiling starches prepared by oxidation, enzyme conversion particularly by α -amylase, acid hydrolysis, heat and or acid dextrinization, thermal and or sheared products are also useful herein.

Plants Producing Starch

The present invention provides plants producing starch described above. The starch producing plants include, but are not limited to maize, potato, wheat, tapioca, rice, cassava, and sorghum.

5 The starch of the present invention can be extracted from corn plants, such as from the endosperm of corn plants. The corn plants that produce starch of the present invention are double mutant plants of the waxy/sugary-2 genotype. The waxy gene is located at Chromosome 9 of corn while the sugary-2 gene is located at Chromosome 6. (See M. G. Nueffer, L. Jones, and M. Zuber, "The Mutants of Maize" (Crop Science Society of America, Madison, WI, 1968), pp.
10 72 and 73.).

The plants of the present invention are homozygous recessive for the starch synthase IIa (*su2*) gene, and either homozygous or heterozygous for a mutated granule-bound starch synthase I (*GBSSI*) gene. The mutated *GBSSI* gene has less *GBSSI* activity than the wild type *GBSSI* gene (*Wx*), but has higher *GBSSI* activity than the recessive, loss-of-function *GBSSI* gene (*wx*).
15 As used herein, such a waxy mutant gene is called an intermediate waxy mutant. In some embodiments, the intermediate waxy mutant has less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% of the wild type *GBSSI* gene expression. In some embodiments, the intermediate waxy mutant has about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% of the wild
20 type *GBSSI* gene expression.

An intermediate *GBSSI* gene having less *GBSSI* activity than the wild type *GBSSI* gene but higher *GBSSI* activity than the recessive, loss-of-function *GBSSI* gene can be either those previously known in the field, or be created by any suitable mutagenesis method.

Mutant alleles such as *wxS* (a.k.a. *wx-Stonor*), *wx-G*, *wx-B5*, and *wx-M* have intermediate
25 *GBSSI* activity. These mutant alleles are due to retrotransposon insertion in the *GBSSI* gene, as described in Wessler and Varagona (PNAS, 82:4177-4181, 1985) and Varagona et al. (The Plant Cell, 4:811-820, 1992). These intermediate alleles result in a waxy protein having less activity than the wild type waxy protein.

Additional mutant alleles having intermediate *GBSSI* activity can be created by suitable
30 methods. For example, retrotransposon mutagenesis can be used to generate a population for

screening corn plants having a mutated GBSSI gene that has intermediate GBSSI activity. In some embodiments, *wxS* (a.k.a. *wx-Stonor*), *wx-G*, *wx-B5*, and *wx-M* can be used as starting materials to produce additional mutant alleles.

Other mutagenesis methods that can be used to produce mutant alleles having intermediate GBSSI activity include, but are not limited to, chemical mutagenesis, radiation mutagenesis, transposon mutagenesis, insertional mutagenesis, signature tagged mutagenesis, site-directed mutagenesis, and natural mutagenesis.

Still in some embodiments, antisense RNA, ribozyme, dsRNAi, RNA interference (RNAi) can be used to produce mutant alleles having intermediate GBSSI activity. Antisense RNA technology involves expressing in, or introducing into, a cell an RNA molecule (or RNA derivative) that is complementary to, or antisense to, sequences found in a particular mRNA in a cell. By associating with the mRNA, the antisense RNA can inhibit translation of the encoded gene product. The use of antisense technology to reduce or inhibit the expression of specific plant genes has been described, for example in European Patent Publication No. 271988, Smith et al., *Nature*, 334:724-726 (1988); Smith et. al., *Plant Mol. Biol.*, 14:369-379 (1990)). A ribozyme is an RNA that has both a catalytic domain and a sequence that is complementary to a particular mRNA. The ribozyme functions by associating with the mRNA (through the complementary domain of the ribozyme) and then cleaving (degrading) the message using the catalytic domain. RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing or transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The RNAi technique is discussed, for example, in Elbashir, et al., *Methods Enzymol.* 26:199 (2002); McManus & Sharp, *Nature Rev. Genetics* 3:737 (2002); PCT application WO 01/75164; Martinez et al., *Cell* 110:563 (2002); Elbashir et al., *supra*; Lagos-Quintana et al., *Curr. Biol.* 12:735 (2002); Tuschl et al., *Nature Biotechnol.* 20:446 (2002); Tuschl, *Chembiochem.* 2:239 (2001); Harborth et al., *J. Cell Sci.* 114:4557 (2001); et al., *EMBO J.* 20:6877 (2001); Lagos-Quintana et al., *Science* 294:8538 (2001); Hutvagner et al., *loc cit*, 834; Elbashir et al., *Nature* 411:494 (2001).

Also within the scope of this invention is the starch resulting from mutants wherein the mutant *wx* and/or *su2* alleles have been moved to another portion of the plant genome by

translocation, inversion, or any other methods of chromosome engineering. In addition, starch extracted from a plant grown from artificial mutations and variations of the above genetic composition which may be produced by known standard methods of mutation breeding is also applicable herein.

5 Also within the scope of this invention is the starch produced from plants other than corn plants, as long as the starch producing plants have a mutant ortholog of the maize GBSSI gene that has intermediate GBSSI activity, and a recessive mutant ortholog of the maize SSIIa gene. In some embodiments, the plants can be wheat, tapioca, rice, cassava, sago, potato, or sorghum.

In some embodiments, the ortholog GBSSI gene in plants other than maize encodes a
10 protein having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more identity to SEQ ID NO: 1, and have the GBSSI activity. In some embodiments, the ortholog has the sequence of UniProt entry number O82627 (*Antirrhinum majus* (Garden
15 snapdragon)), P12615 (*Avena sativa* (Oat)), Q42968 (*Oryza glaberrima* (African rice)), Q43784 (*Manihot esculenta* (Cassava)), P0C585 (*Oryza sativa* (Rice)), Q00775 (*Solanum tuberosum* (Potato)), P27736 (*Triticum aestivum* (Wheat)), Q43134 (*Sorghum bicolor* (Sorghum)), Q43092 (*Pisum sativum* (Garden pea)), P84766 (*Aegilops tauschii* subsp. *strangulata* (Goatgrass)), Q0DEV5 (*Oryza sativa* subsp. *japonica* (Rice)), Q42857 (*Ipomoea batatas* (Sweet potato)),
20 P84633 (*Fagopyrum esculentum* (Common buckwheat)), P84765 (*Secale cereale* (Rye)), Q9MAQ0 (*Arabidopsis thaliana*), P09842 (*Hordeum vulgare* (Barley)), or A2Y8X2 (*Oryza sativa* subsp. *indica* (Rice)), each of which is herein incorporated by reference in its entirety.

In some embodiments, the ortholog SSIIa gene in plants other than maize encodes a
25 protein having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more identity to SEQ ID NO: 2, and have the SSIIa activity. In some embodiments, the ortholog has the sequence of UniProt entry number V6BPG5 (*Triticum urartu* (Red wild einkorn)), V6BPN2 (*Triticum monococcum* subsp. *Monococcum*), C3W8L4 (*Hordeum vulgare* var. *distichum* (Domesticated barley)), E2GHR4 (*Oryza sativa* subsp. *indica* (Rice)), or E2GHR6
30

(*Oryza sativa* subsp. *japonica* (Rice)), each of which is herein incorporated by reference in its entirety.

In some embodiments, to produce double mutant plants having the intermediate *wx* mutant and the recessive *su2* mutant, a cross pollination between a plant having the intermediate *wx* mutant and a plant having the recessive *su2* mutant can be made.

For example, a cross pollination can be made between a homozygous *wxS* mutant (*wxSwxSSU2SU2*) or a heterozygous *wxS* mutant (*wxSWxSU2SU2*) and a homozygous *waxy su2* mutant (*wxwxsu2su2*) or a heterozygous *waxy su2* mutant (*wxWxsu2su2* or *wxwxSU2su2*). After the cross, self pollination of the F1 generation having the genotype of *wxSwxSU2su2* can be used to generate the F2 plants. Plants having either zero, one, two, or three doses of the *wxS* gene in the endosperm, and three doses of the recessive *su2* gene can be isolated accordingly (e.g., endosperm having the genotype of *wxwxwxsu2su2su2*, *wxSwxwxsu2su2su2*, *wxSwxSwxsu2su2su2*, or *wxSwxSwSsu2su2su2*). This is only one example of the breeding method, and other breeding schemes can be used, and are within the scope of the present invention.

In some embodiments, field production of plants with endosperms that have at least one dose of the intermediate *waxy* allele and the recessive *sugary-2* allele is carried out by crossing female maize plants with male maize plants. A typical planting arrangement is one male row to several female rows. The female rows are either detasseled or rendered male sterile through various other means known in the art such as cytoplasmic or genetic means.

The starch utilized in this invention may be obtained from inbred lines, or the starch can be obtained from hybrids derived from inbreds containing the intermediate *waxy*/ recessive *su2* double mutant. While maize is the exemplary plant herein for the source of the *waxy* starch, the invention is also applicable to other plant species such as, for example, *waxy* rice, *waxy* barley and *waxy* sorghum, provided that they have an intermediate *waxy* gene and a recessive *su2* gene.

Breeding Methods

To make the plants of the present invention, suitable breeding or selection methods can be used. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, recurrent selection, and backcross breeding.

Open-Pollinated Populations. The improvement of open-pollinated populations of such crops as rye, many maizes and sugar beets, herbage grasses, legumes such as alfalfa and clover, and tropical tree crops such as cacao, coconuts, oil palm and some rubber, depends essentially upon changing gene-frequencies towards fixation of favorable alleles while maintaining a high (but far from maximal) degree of heterozygosity. Uniformity in such populations is impossible and trueness-to-type in an open-pollinated variety is a statistical feature of the population as a whole, not a characteristic of individual plants. Thus, the heterogeneity of open-pollinated populations contrasts with the homogeneity (or virtually so) of inbred lines, clones and hybrids.

Population improvement methods fall naturally into two groups, those based on purely phenotypic selection, normally called mass selection, and those based on selection with progeny testing. Interpopulation improvement utilizes the concept of open breeding populations; allowing genes to flow from one population to another. Plants in one population (cultivar, strain, ecotype, or any germplasm source) are crossed either naturally (e.g., by wind) or by hand or by bees (commonly *Apis mellifera* L. or *Megachile rotundata* F.) with plants from other populations. Selection is applied to improve one (or sometimes both) population(s) by isolating plants with desirable traits from both sources.

There are basically two primary methods of open-pollinated population improvement. First, there is the situation in which a population is changed en masse by a chosen selection procedure. The outcome is an improved population that is indefinitely propagable by random-mating within itself in isolation. Second, the synthetic variety attains the same end result as population improvement but is not itself propagable as such; it has to be reconstructed from parental lines or clones. These plant breeding procedures for improving open-pollinated populations are well known to those skilled in the art and comprehensive reviews of breeding procedures routinely used for improving cross-pollinated plants are provided in numerous texts and articles, including: Allard, *Principles of Plant Breeding*, John Wiley & Sons, Inc. (1960); Simmonds, *Principles of Crop Improvement*, Longman Group Limited (1979); Hallauer and Miranda, *Quantitative Genetics in Maize Breeding*, Iowa State University Press (1981); and, Jensen, *Plant Breeding Methodology*, John Wiley & Sons, Inc. (1988).

Mass Selection. In mass selection, desirable individual plants are chosen, harvested, and the seed composited without progeny testing to produce the following generation. Since

selection is based on the maternal parent only, and there is no control over pollination, mass selection amounts to a form of random mating with selection. As stated herein, the purpose of mass selection is to increase the proportion of superior genotypes in the population.

Synthetics. A synthetic variety is produced by crossing inter se a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination. Whether parents are (more or less inbred) seed-propagated lines, as in some sugar beet and beans (*Vicia*) or clones, as in herbage grasses, clovers and alfalfa, makes no difference in principle. Parents are selected on general combining ability, sometimes by test crosses or topcrosses, more generally by polycrosses. Parental seed lines may be deliberately inbred (e.g. by selfing or sib crossing). However, even if the parents are not deliberately inbred, selection within lines during line maintenance will ensure that some inbreeding occurs. Clonal parents will, of course, remain unchanged and highly heterozygous.

Whether a synthetic can go straight from the parental seed production plot to the farmer or must first undergo one or two cycles of multiplication depends on seed production and the scale of demand for seed. In practice, grasses and clovers are generally multiplied once or twice and are thus considerably removed from the original synthetic.

While mass selection is sometimes used, progeny testing is generally preferred for polycrosses, because of their operational simplicity and obvious relevance to the objective, namely exploitation of general combining ability in a synthetic.

The number of parental lines or clones that enter a synthetic vary widely. In practice, numbers of parental lines range from 10 to several hundred, with 100-200 being the average. Broad based synthetics formed from 100 or more clones would be expected to be more stable during seed multiplication than narrow based synthetics.

Pedigreed varieties. A pedigreed variety is a superior genotype developed from selection of individual plants out of a segregating population followed by propagation and seed increase of self pollinated offspring and careful testing of the genotype over several generations. This is an open pollinated method that works well with naturally self pollinating species. This method can be used in combination with mass selection in variety development. Variations in pedigree and mass selection in combination are the most common methods for generating varieties in self pollinated crops.

Hybrids. A hybrid is an individual plant resulting from a cross between parents of differing genotypes. Commercial hybrids are now used extensively in many crops, including corn (maize), sorghum, sugarbeet, sunflower and broccoli. Hybrids can be formed in a number of different ways, including by crossing two parents directly (single cross hybrids), by crossing a single cross hybrid with another parent (three-way or triple cross hybrids), or by crossing two different hybrids (four-way or double cross hybrids).

Pedigree Selection. Pedigree breeding is used commonly for the improvement of self-pollinating crops or inbred lines of cross-pollinating crops. Two parents possessing favorable, complementary traits are crossed to produce an F_1 . An F_2 population is produced by selfing one or several F_1 s or by intercrossing two F_1 s (sib mating). The dihaploid breeding method or other ploidy reduction techniques could also be used. Selection of the best individuals is usually begun in the F_2 population; then, beginning in the F_3 , the best individuals in the best families are selected. Replicated testing of families, or hybrid combinations involving individuals of these families, often follows in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release of new cultivars. Similarly, the development of new cultivars through the dihaploid system requires the selection of the cultivars followed by two to five years of testing in replicated plots.

Backcross breeding. Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent.

Bulk Segregation Analysis (BSA). BSA, a.k.a. bulked segregation analysis, or bulk segregant analysis, is a method described by Michelmore *et al.* (Michelmore *et al.*, 1991, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations.

Proceedings of the National Academy of Sciences, USA, 99:9828-9832) and Quarrie *et al.* (Quarrie *et al.*, 1999, *Journal of Experimental Botany*, 50(337):1299-1306).

For BSA of a trait of interest, parental lines with certain different phenotypes are chosen and crossed to generate F2, doubled haploid or recombinant inbred populations with QTL analysis. The population is then phenotyped to identify individual plants or lines having high or low expression of the trait. Two DNA bulks are prepared, one from the individuals having one phenotype (e.g., resistant to virus), and the other from the individuals having reversed phenotype (e.g., susceptible to virus), and analyzed for allele frequency with molecular markers. Only a few individuals are required in each bulk (e.g., 10 plants each) if the markers are dominant (e.g., RAPDs). More individuals are needed when markers are co-dominant (e.g., RFLPs). Markers linked to the phenotype can be identified and used for breeding or QTL mapping.

Targeting Induced Local Lesions in Genomes (TILLING®). Breeding schemes of the present application can include crosses with TILLING® plant cultivars. TILLING® is a method in molecular biology that allows directed identification of mutations in a specific gene. TILLING® was introduced in 2000, using the model plant *Arabidopsis thaliana*. TILLING® has since been used as a reverse genetics method in other organisms such as zebrafish, corn, wheat, rice, soybean, tomato and Brassica rapa var. nipposinica.

The method combines a standard and efficient technique of mutagenesis with a chemical mutagen (e.g., Ethyl methanesulfonate (EMS)) with a sensitive DNA screening-technique that identifies single base mutations (also called point mutations) in a target gene. EcoTILLING is a method that uses TILLING® techniques to look for natural mutations in individuals, usually for population genetics analysis (*see* Comai, *et al.*, 2003 *The Plant Journal* 37, 778-786; Gilchrist *et al.* 2006 *Mol. Ecol.* 15, 1367-1378; Mejlhede *et al.* 2006 *Plant Breeding* 125, 461-467; Nieto *et al.* 2007 *BMC Plant Biology* 7, 34-42, each of which is incorporated by reference hereby for all purposes). DEcoTILLING is a modification of TILLING® and EcoTILLING which uses an inexpensive method to identify fragments (Garvin *et al.*, 2007, DEco-TILLING: An inexpensive method for SNP discovery that reduces ascertainment bias. *Molecular Ecology Notes* 7, 735-746).

The TILLING® method relies on the formation of heteroduplexes that are formed when multiple alleles (which could be from a heterozygote or a pool of multiple homozygotes and

heterozygotes) are amplified in a PCR, heated, and then slowly cooled. A “bubble” forms at the mismatch of the two DNA strands (the induced mutation in TILLING® or the natural mutation or SNP in EcoTILLING), which is then cleaved by single stranded nucleases. The products are then separated by size on several different platforms.

5 More detailed description on methods and compositions on TILLING® can be found in US 5994075, US 2004/0053236 A1, WO 2005/055704, and WO 2005/048692, each of which is hereby incorporated by reference for all purposes.

Thus in some embodiments, the breeding methods of the present disclosure include breeding with one or more TILLING® plant lines with one or more identified mutations.

10 Mutation Breeding. Mutation breeding is another method of introducing new traits into plants. Mutations that occur spontaneously or are artificially induced can be useful sources of variability for a plant breeder. The goal of artificial mutagenesis is to increase the rate of mutation for a desired characteristic. Mutation rates can be increased by many different means or mutating agents including temperature, long-term seed storage, tissue culture conditions,
15 radiation (such as X-rays, Gamma rays, neutrons, Beta radiation, or ultraviolet radiation), chemical mutagens (such as base analogs like 5-bromo-uracil), antibiotics, alkylating agents (such as sulfur mustards, nitrogen mustards, epoxides, ethyleneamines, sulfates, sulfonates, sulfones, or lactones), azide, hydroxylamine, nitrous acid or acridines. Once a desired trait is observed through mutagenesis the trait may then be incorporated into existing germplasm by
20 traditional breeding techniques. Details of mutation breeding can be found in W. R. Fehr, 1993, Principles of Cultivar Development, Macmillan Publishing Co. New breeding techniques such as the ones involving the uses of Zinc Finger Nucleases or oligonucleotide directed mutagenesis shall also be used to generate genetic variability and introduce new traits into varieties.

In some embodiments, the mutation breeding comprises a single locus conversion. A
25 single locus may contain several genes and/or transgenes, such as a transgene for disease resistance that, in the same expression vector, also contains a transgene for herbicide resistance. The gene for herbicide resistance may be used as a selectable marker and/or as a phenotypic trait. A single locus conversion of site specific integration system allows for the integration of multiple genes at the converted locus. A single locus conversion also allows for making one or
30 more site specific changes to the plant genome. In some embodiments, the single locus

conversion is performed by genome editing, a.k.a. genome editing with engineered nucleases (GEEN). In some embodiments, the genome editing comprises using one or more engineered nucleases. In some embodiments, the engineered nucleases include, but are not limited to Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, and engineered meganuclease re-engineered homing endonucleases. In some embodiments, the single locus conversion changes one or several nucleic acids of the plant genome.

Double Haploids and Chromosome Doubling. One way to obtain homozygous plants without the need to cross two parental lines followed by a long selection of the segregating progeny, and/or multiple back-crossings is to produce haploids and then double the chromosomes to form doubled haploids. Haploid plants can occur spontaneously, or may be artificially induced via chemical treatments or by crossing plants with inducer lines (Seymour *et al.* 2012, PNAS vol 109, pg 4227-4232; Zhang *et al.*, 2008 Plant Cell Rep. Dec 27(12) 1851-60). The production of haploid progeny can occur via a variety of mechanisms which can affect the distribution of chromosomes during gamete formation. The chromosome complements of haploids sometimes double spontaneously to produce homozygous doubled haploids (DHs). Mixoploids, which are plants which contain cells having different ploidies, can sometimes arise and may represent plants that are undergoing chromosome doubling so as to spontaneously produce doubled haploid tissues, organs, shoots, floral parts or plants. Another common technique is to induce the formation of double haploid plants with a chromosome doubling treatment such as colchicine (El-Hennawy *et al.*, 2011 Vol 56, issue 2 pg 63-72; Doubled Haploid Production in Crop Plants 2003 edited by Maluszynski ISBN 1-4020-1544-5). The production of doubled haploid plants yields highly uniform cultivars and is especially desirable as an alternative to sexual inbreeding of longer-generation crops. By producing doubled haploid progeny, the number of possible gene combinations for inherited traits is more manageable. Thus, an efficient doubled haploid technology can significantly reduce the time and the cost of inbred and cultivar development.

Protoplast Fusion. In another method for breeding plants, protoplast fusion can also be used for the transfer of trait-conferring genomic material from a donor plant to a recipient plant. Protoplast fusion is an induced or spontaneous union, such as a somatic hybridization, between

two or more protoplasts (cells of which the cell walls are removed by enzymatic treatment) to produce a single bi- or multi-nucleate cell. The fused cell that may even be obtained with plant species that cannot be interbred in nature is tissue cultured into a hybrid plant exhibiting the desirable combination of traits.

5 Tissue Culture. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that can generate tissue culture that are intact in plants or parts of plants, such as embryos, pollen, flowers, seeds, leaves, stems, roots, root tips, anthers, pistils, meristematic cells, axillary buds, ovaries, seed coat, endosperm, hypocotyls, cotyledons and the like. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs has been used to produce regenerated plants. U.S. Patent Nos. 5,959,185, 5,973,234, and 5,977,445 describe certain techniques, the disclosures of which are incorporated herein by reference.

15 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

20 EXAMPLE 1

Generating grain with 1, 2, or 3 doses of the *wxS* allele

Because of the triploid nature of the corn endosperm, wherein two polar nuclei from the maternal parent are fertilized by one sperm cell from the paternal parent, three genotypic combinations are possible. Using a *wxSwxSsu2su2* plant as a female, pollinated by a *wxwxsu2su2* male results in 'dose-2' or *wxSwxSwxsu2su2su2* grain. Using the *wxSwxSsu2su2* as a male, pollinating a *wxwxsu2su2* female results in 'dose-1' or *wxSwxwxsu2su2su2* grain. Self pollination of *wxSwxSsu2su2* results in 'dose-3' or *wxSwxSwxSsu2su2su2* grain.

<i>wxS</i> dosage	Endosperm Genotype
(1 <i>wxS</i>)	<i>wxSwxwxsu2su2su2</i>
(2 <i>wxS</i>)	<i>wxSwxSwxsu2su2su2</i>
(3 <i>wxS</i>)	<i>wxSwxSwxSsu2su2su2</i>
starch from <i>wxwxwxsu2su2su2</i> seed (0 <i>wxS</i>)	<i>wxwxwxsu2su2su2</i>
AMIOCA™ starch (0 <i>wxS</i>)	<i>wxwxwxSU2SU2SU2</i>
starch from <i>wxwxwxSU2su2su2</i> (0 <i>wxS</i>)	<i>wxwxwxSU2su2su2</i>

EXAMPLE 2

Viscosity of Starch Using a Rapid Visco Amylograph (RVA)

5 The viscosity of maize starch derived from plants containing 0, 1, 2, and 3 doses of the *wxS* allele in the recessive sugary-2 mutant plants was determined using a Rapid Visco Amylograph (Newport Scientific, Eden Prairie, Minn.). The AMIOCA™ starch and *wxwxwxSU2su2su2* starch are included as checks. The genotype of endosperms from which the starch was isolated is summarized in the table below. The amylose content was measured using

10 Megazyme® amylose kit. The amylose content of the starch increases with the increase of GBSSI activity. The amylose contents of *wxwxwxsu2su2su2* starch (7.4%), AMIOCA™ starch (7.1%), and *wxwxwxSU2su2su2* starch (6.1%) were lower than Waxy Stonor (12.8%) and *wxwxwxsu2su2su2* starch with 1 to 3 doses of *wxS* gene in the endosperm (8.2-14.6%). The amylose content of *wxwxwxsu2su2su2* starch (7.4%, 8.2%, 13.8%, and 14.6%) increased with the

15 increase of *wxS* dosage (0, 1, 2, and 3 doses of *wxS*, respectively). The results suggested that the introduction of *wxS* gene to *wxwxwxsu2su2su2* slightly increased amylose content of the starch.

Sample	Amylose (%)
Q3017a (1 wxS)	8.2±0.1
Q8412a (2 wxS)	13.8±0.0
Q7738a (3 wxS)	14.6±0.1
<i>wxwxwxsu2su2su2</i> starch (0 wxS)	7.4±0.1
K5594a (Waxy Stonor)	12.8±0.1
AMIOCA™ starch	7.1±0.0
<i>wxwxwxSU2su2su2</i> starch	6.1±0.1

Pasting property of waxy corn starch was analyzed using a Rapid Visco-Analyzer (RVA, Newport Scientific, Sydney, Australia) using “Standard 2” profile method. A suspension (28.0 g) containing 8% starch (w/w, dry starch basis, dsb) was equilibrated at 50°C for 1 min, heated to 95 °C at a rate of 6 °C/min, held at 95°C for 5 min, and then cooled to 50°C at a rate of 6 °C/min. The rotating speed for the paddle was 160 rpm except that 960 rpm was used for the first 10 s. The results are shown in FIG. 1 and FIG. 2. The existing *wxwxwxsu2su2su2* starch had the lowest pasting temperature, peak viscosity, and final viscosity. After introduction of wxS gene to *wxwxwxsu2su2su2* starch, the starch samples (Q3017a [1 wxS], Q8412a [2 wxS], and Q7738a [3 wxS]) showed increased peak viscosity compared to the existing *wxwxwxsu2su2su2* starch (0 wxS). However, only the *wxSwxwxsu2su2su2* starch with one dose of wxS gene showed the peak viscosity much higher than Waxy Stonor, AMIOCA™ starch, and *wxwxwxSU2su2su2* starch.

The results indicate that improvement in pasting temperature will allow starch extraction via the wet-milling process.

EXAMPLE 3

Freeze-thaw Stability of Starch

The maize starch derived from plants containing 0, 1, 2, and 3 doses of the wxS allele in the recessive sugary-2 mutant plants was tested for freeze-thaw stability. AMIOCA™ starch and *wxwxwxSU2su2su2* starch were included as checks. Starch samples 8% solids were cooked in a water bath at 95° C for 20 minutes. The starch slurry was mixed with a glass rod for three minutes followed by quiescent heating for 17 minutes during cooking. Freeze-thaw stability of starch gel (8%, w/w) at neutral pH was analyzed using a Caron cycling freezer for five freeze-thaw cycles. In each cycle, the sample was frozen for 6 hours and thawed for 6 hours with 2

cycles / day. The samples were visually inspected for syneresis, both on the surface and when pressed, as well as for opacity and gelling changes. This freeze-thaw cycle was repeated. The results are shown in FIG. 3. After first freeze/thaw cycle, only Waxy Stonor starch gel showed high opacity, indicating high degree of starch retrogradation. The opacity of starch gel increased with the increase of freeze/thaw cycles. At third freeze/thaw cycle, the starch gels of AMIOCA™ starch, *wxwxwxSU2su2su2* starch, and Waxy Stonor became very opaque, whereas the starch gels of Q3017a (1 wxS), Q8412a (2 wxS), Q7738a (3 wxS), and existing *wxwxwxsu2su2su2* starch were still not opaque. After five freeze/thaw cycles, Q3017a (1 wxS), Q8412a (2 wxS), Q7738a (3 wxS), and existing *wxwxwxsu2su2su2* starch become slightly opaque.

The results suggested that the slight increase of amylose in starch samples had little effect in the freeze/thaw stability. The Q3017a (1 wxS), Q8412a (2 wxS), Q7738a (3 wxS) starches had freeze/thaw stability better than *wxwxwxSU2su2su2* starch and AMIOCA™ starch.

EXAMPLE 4

Differential Scanning Calorimetry (DSC) Test

Thermal properties of maize starch derived from plants containing 0, 1, 2, and 3 doses of the *wxS* allele in the recessive sugary-2 mutant plants were determined using Differential Scanning Calorimetry (DSC). The AMIOCA™ starch and *wxwxwxSU2su2su2* starch were included as checks. Differential Scanning Calorimetry (DSC) measures heat absorbed or given off by a sample in a controlled atmosphere at specified temperatures. DSC provides information about a specific heat and latent heat of samples as temperature rises, which indicates changes in the amorphous and crystalline structures. Data is recorded in terms of heat flow, and is presented in joules/gram (J/g) (Cassel, 2002). In the analysis of starch, starch gelatinization parameters such as peak onset, peak temperature, end of peak, and gelatinization enthalpy change information were collected. The results are shown in FIG. 4. *wxSwxwxsu2su2su2* starch (Q3017a), *wxSwxSwxsu2su2su2* starch (Q8412a), *wxSwxSwxSsu2su2su2* starch (Q7738a), and *wxwxwxsu2su2su2* starch (0 wxS) showed lower gelatinization temperatures than AMIOCA™ starch, Waxy Stonor, and *wxwxwxSU2su2su2* starch, which is consistent with the better

freeze/thaw stability of the starch isolated from endosperm of maize with one, two, and three doses of *wxS*. The *wxSwxwxsu2su2su2* starch with one dose of *wxS* (Q3017a) had slightly higher onset gelatinization temperature than other hybrids (Q8412a [2 *wxS*], Q7738a [3 *wxS*], and *wxwxwxsu2su2su2* starch), indicating that the Q3017a had better wet milling characteristics than other hybrids.

EXAMPLE 5

Starch Granule

The average particle size of waxy maize starch derived from plants containing 1, 2, and 3 doses of the *wxS* allele in the recessive sugary-2 mutant plants was measured by laser light scattering. The AMIOCA™ starch and *wxwxwxSU2su2su2* starch were included as checks. The results are shown in FIG. 5. In addition, the Scanning electron micrograph of each sample was provided in FIG. 6 to show granule morphology. The average starch granule sizes of Q3017a (11.4μm), Q8412a (11.4μm), and Q7738a (10.9μm) were larger than the existing *wxwxwxsu2su2su2* starch (10.4 μm), suggesting that the introduction of *wxS* gene to Waxy VO increased the starch granule size. The result was consistent with the SEM images (Figure 5) showing that the introduction of *wxS* gene to *wxwxwxsu2su2su2* starch increased the starch granule integrity.

Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present disclosure, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior invention.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to

cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

5

35

130416790 v3

WHAT IS CLAIMED IS:

1. A starch comprising:
an amylose content of from about 2 wt % to about 20 wt % as measured using an amylose/amylopectin assay kit; and
5 an aqueous starch pasting temperature that is at least about 5% greater than the starch pasting temperature of a control starch as measured using RVA analysis,
wherein, the starch is derived from a waxy-sugary 2 corn plant comprising at least one *wxS* allele in the endosperm.
- 10 2. The starch of claim 1, wherein the amylose content is from about 8 wt % to about 15 wt %.
3. The starch of any of claims 1 or 2, wherein the aqueous pasting temperature is from about 5 % to about 14 % greater than that of the control starch.
- 15 4. The starch of any of claims 1-3, further comprising an enthalpy change of crystallinity of at least about 30 % greater than an enthalpy change of crystallinity of the control starch as measured using RVA analysis.
- 20 5. The starch of claim 4, wherein the enthalpy change of crystallinity is from about 70 % to about 200 % greater than an enthalpy change of crystallinity of the control starch as measured using a differential scanning calorimeter (DSC).
- 25 6. The starch of any of claims 1-4, further comprising a distribution of starch granule sizes, wherein the distribution of starch granule sizes comprises at least about 40 % fewer granules less than 5 microns compared to a proportion of granules less than 5 microns of the control starch as observed using a scanning electron microscope.
- 30 7. The starch of any of claims 1-6, wherein the starch is a granule which exhibits increased integrity compared to the control starch as observed using a scanning electron microscope.

8. The starch of any of claims 1-7, wherein the starch is capable of withstanding at least three freeze-thaw cycles.

9. The starch of claim 9, wherein the starch is capable of withstanding at least three to at least five freeze-thaw cycles.

10. The starch of any of claims 1-9, wherein the waxy-sugary 2 corn plant is generated by cross pollination of a first corn plant having the genotype of *wxwx su2 su2*, and a second corn plant having the genotype of *wxS wxS su2 su2*, or the genotype of *wxS wx su2 su2*.

11. The starch of any of claims 1-10, wherein the endosperm of the waxy-sugary 2 corn plant has one dose, two doses, or three doses of the *wxS* allele, and the endosperm has the genotype of *wxS wx wx su2 su2 su2*, *wxS wx S wx su2 su2 su2*, or *wxS wx S wx S su2 su2 su2*.

12. A method for improving at least one characteristics in a pasting profile of a starch comprising introducing at least one *wxS* allele into the endosperm of a waxy corn plant, wherein the resultant starch derived from the waxy corn plant comprising at least one *wxS* allele in the endosperm comprises an aqueous starch pasting temperature which is at least about 5% greater than a starch pasting temperature of a control starch as measured using RVA analysis, thereby improving at least one characteristic of the pasting profile of the starch compared a control starch

13. A double-mutant corn plant, wherein the corn plant is homozygous recessive for the starch synthase IIa (*su2*) gene, and either homozygous or heterozygous for a mutated Granule-bound starch synthase I (GBSSI) gene, wherein the mutated GBSSI gene has less GBSSI activity than the wild type GBSSI gene (*Wx*), but has higher GBSSI activity than the recessive, loss-of-function GBSSI gene (*wx*).

14. The double-mutant corn plant of claim 13, wherein the mutated GBSSI gene is the *wx-Stonor* (*wxS*) allele.

15. The double-mutant corn plant of claim 14, wherein the plant is generated by cross pollination of a first corn plant having the genotype of *wxwxsu2su2*, and a second corn plant having the genotype of *wxSwxSsu2su2*, or the genotype of *wxSwxsu2su2*.

5 16. The double-mutant corn plant of claim 15, wherein the endosperm of the corn plant has one dose, two doses, or three doses of the *wxS* allele, wherein the endosperm has the genotype of *wxSwxwxsu2su2su2*, *wxSwxSwxsu2su2su2*, or *wxSwxSwxSsu2su2su2*.

10 17. The double-mutant corn plant of any of claims 13-16, wherein the starch produced in the endosperm of the corn plant has a higher pasting temperature compared to the starch produced in the endosperm of a double, waxy sugary-2 recessive mutant corn plant having the genotype of *wxwxwxsu2su2su2*.

15 18. A seed of the double-mutant corn plant of any of claims 13-17.

19. A plant part, plant cell, or tissue culture of the double-mutant corn plant of any of claims 13-18.

20 20. The plant part of claim 19, wherein the plant part is selected from the group consisting of an endosperm, a leaf, a flower, an ovule, a pollen, a rootstock, and a scion.

21. A method for producing starch comprising obtaining starch from the endosperm of the double-mutant corn plant of any of claims 13-20.

25 22. A method for producing starch comprising growing the double-mutant corn plant of any of claims 13-21, and harvesting seeds from the double-mutant corn plant.

30 23. A method for producing a corn seed comprising crossing a first parent corn plant with a second parent corn plant and harvesting the resultant corn seed, wherein said first parent corn plant and/or second parent corn plant is the double-mutant corn plant of any of claims 13-22.

24. A method for producing a corn seed comprising self-pollinating the double-mutant corn plant of any of claims 13-23, and harvesting the resultant corn seed.

5 25. A method of vegetatively propagating the corn plant of any of claims 13-24, said method comprising collecting part of the plant of any of claims 1-11 and regenerating a plant from said part.

10 26. A method of producing a corn plant derived from the double-mutant corn plant of any of claims 13-17, the method comprising self-pollinating the double-mutant plant of any of claims 13-17 at least once to produce a progeny plant derived from the double-mutant plant of any of claims 13-17.

15 27. A method of producing a corn plant derived from the double-mutant plant of any of claims 1-11, the method comprising crossing the double-mutant plant of any of claims 13-17 with a second corn plant to produce a progeny plant the double-mutant plant of any of claims 13-17.

20 28. A method of producing a corn plant comprising introducing a transgene into a corn plant, wherein the transgene confers the desired phenotype of the double-mutant plant of any of claims 13-17.

25 29. A method of producing a corn plant comprising gene-editing to produce a genetic sequence to confer the desired phenotype of the double-mutant plant of any of claims 13-17.

30 30. A method of producing a corn plant comprising introducing a mutation to produce a genetic sequence to confer the desired phenotype of the double-mutant plant of any of claims 13-17.

30 31. A starch extracted from the double-mutant corn plant of any of claims 13-17.

32. A starch extracted from the double-mutant corn plant of claim 15.
33. A starch extracted from the endosperm of the double-mutant corn plant of claim 16.

5

ABSTRACT OF THE DISCLOSURE

5 This invention disclosure relates to novel maize starch. The starch can be made from
the newly developed waxy sugary-2 double-mutant maize that has low activity of Granule Bound
Starch Synthase I (GBSSI), which results in low amylose level. The starch from newly
developed waxy sugary-2 double-mutant is freeze-thaw stable and has high viscosity. In
comparison with the starch of the existing waxy sugary-2 double-mutant maize, the new waxy
sugary-2 double-mutant maize starch showed, *inter alia*, improved pasting profile, starch granule
10 integrity, larger starch granule size, and higher viscosity.

FIG. 1
Starch pasting profiles of waxy corn starch samples.

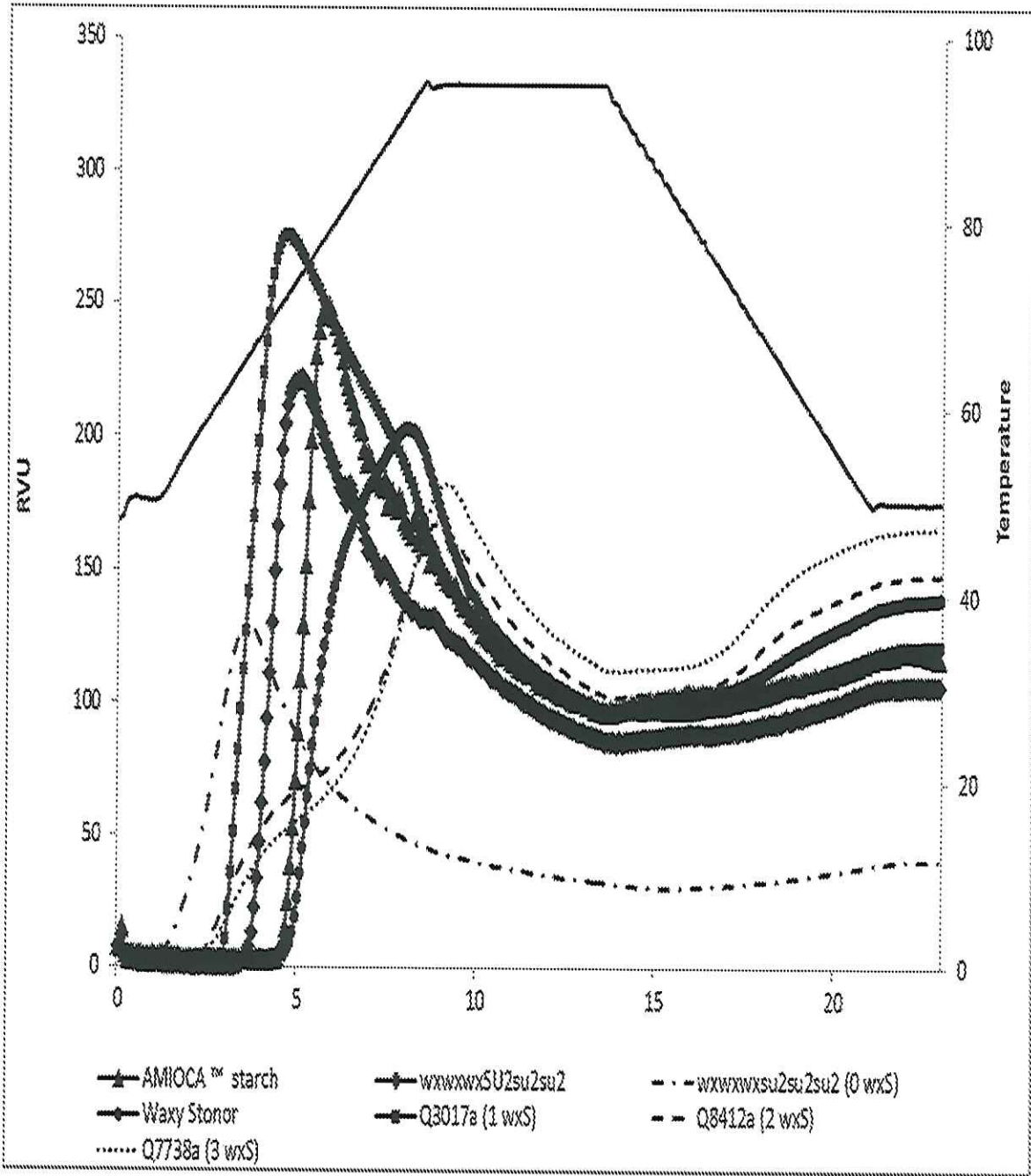
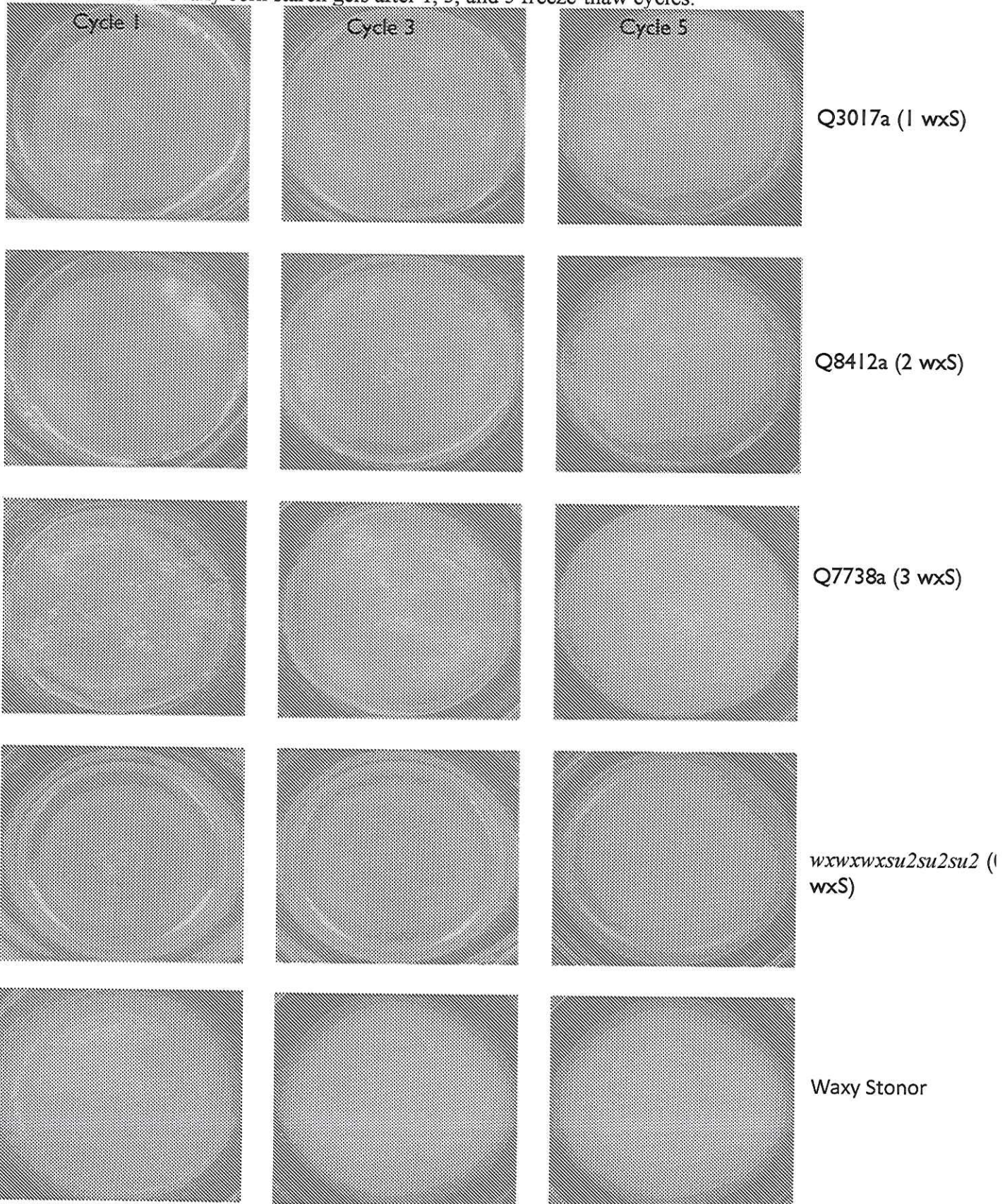


FIG. 2
Starch pasting properties of waxy corn starch samples.

Sample	Pasting Temp. (°C)	Peak (RVU)	Hold (RVU)	Final (RVU)	Break Down (RVU)	Setback (RVU)
Q3017a (1 wxS)	62.0	275.7	95.3	121.3	180.4	26.0
Q8412a (2 wxS)	59.1	167.8	102.1	148.0	65.7	45.9
Q7738a (3 wxS)	59.5	182.2	111.9	165.6	70.2	53.7
<i>wxwxwxsu2su2su2</i> (0 wxS)	54.4	129.3	30.3	40.9	99.0	10.6
K5994a (waxy Stonor)	73.8	202.6	96.3	139.0	106.3	42.7
AMIOCA™ starch	72.2	248.2	96.3	116.8	151.9	20.5
<i>wxwxwxSU2su2su2</i> starch	67.5	220.9	84.9	106.1	136.0	21.2

FIG. 3. Photos of waxy corn starch gels after 1, 3, and 5 freeze-thaw cycles.



130416790 v3

FIG. 3 Continued

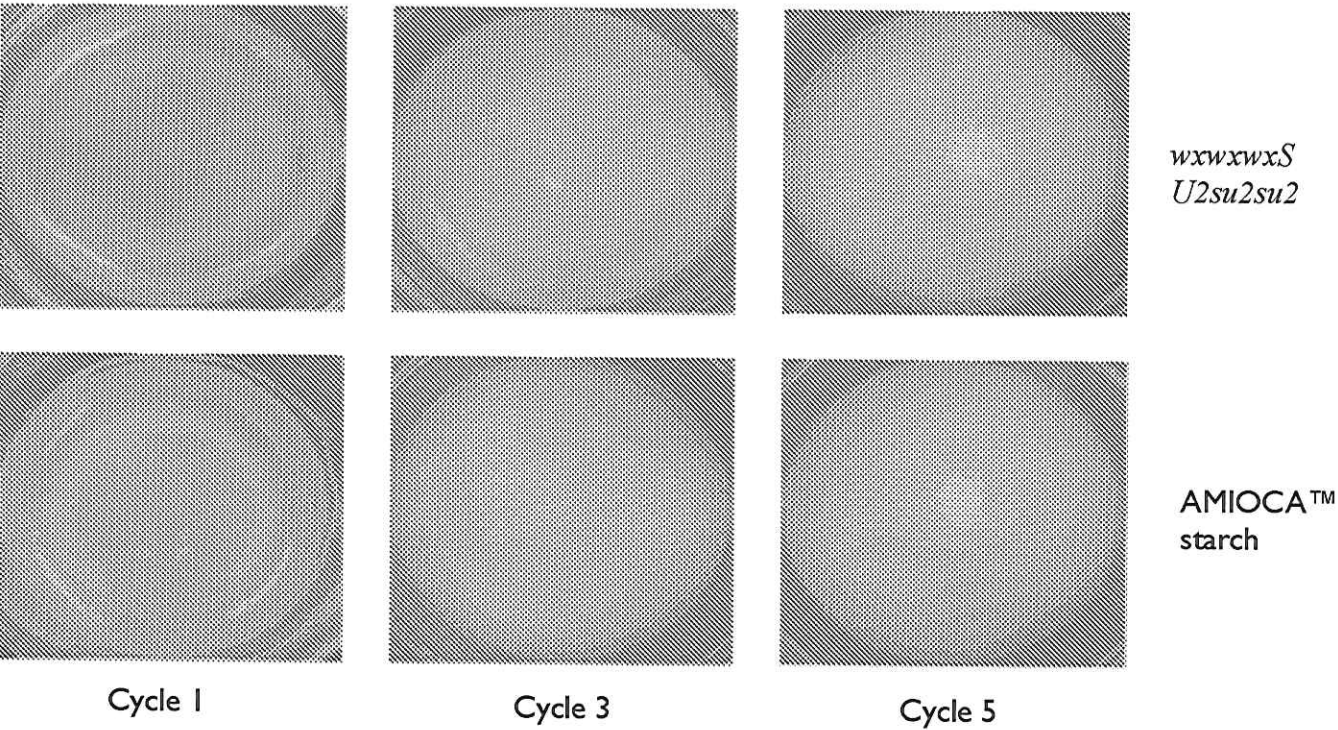
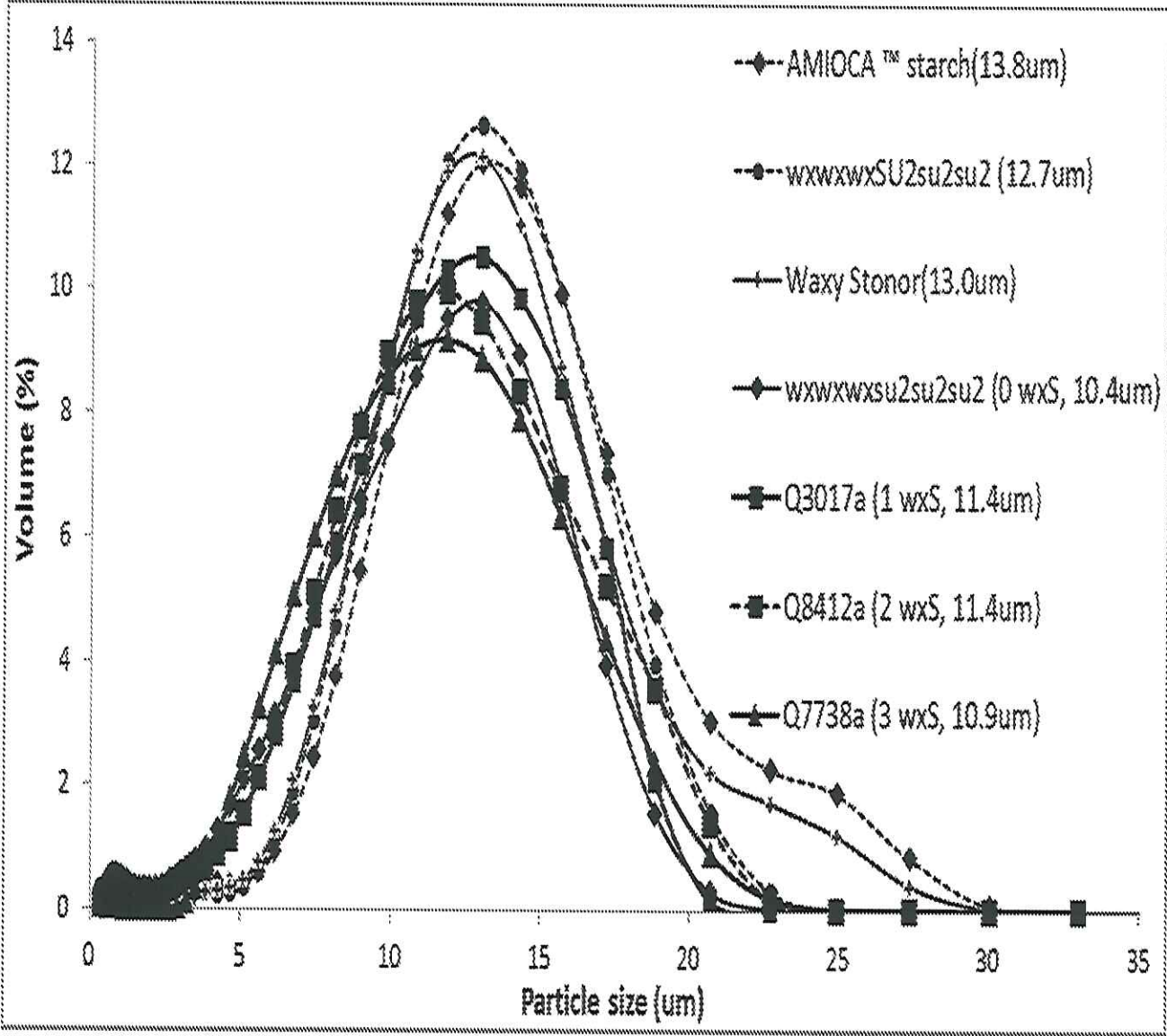


FIG. 4

Thermal Properties of waxy corn starch samples.

Sample	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
Q3017a (1 wxS)	57.2±0.0	62.5±0.3	69.3±0.2	12.9±0.0
Q8412a (2 wxS)	51.2±0.1	58.0±0.1	65.7±0.4	6.0±0.1
Q7738a (3 wxS)	51.7±0.2	58.2±0.4	66.8±0.3	8.0±0.1
wxwxwxsu2su2su2	52.2±0.2	58.1±0.2	65.6±0.0	4.5±0.0
K5994a (Waxy Stonor)	68.1	74.6	81.3	14.9
AMIOCA™ starch	66.7±0.5	73.5±0.3	80.6±0.6	16.6±0.2
wxwxwxSU2su2su2 starch	60.8±0.1	67.8±0.1	77.6±0.2	15.4±0.0

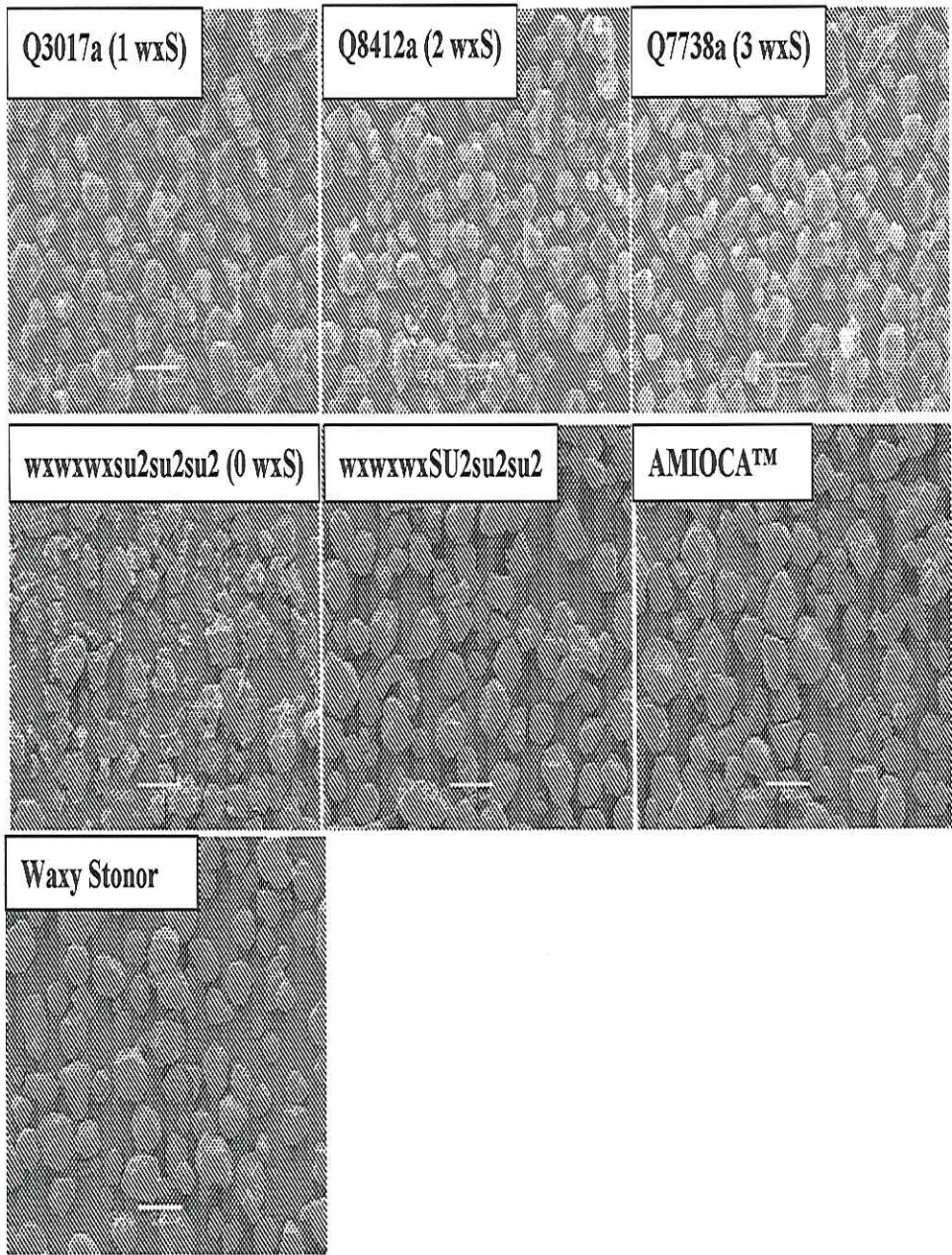
FIG. 5
Granule size distributions of waxy corn starch samples.



5

FIG. 6

Granule morphology of waxy corn starch samples.



Sequence Listing

SEQ ID NO: 1 Waxy Protein in maize					
	10	20	30	40	50
5	MAALATSQLV	ATRAGLGVPD	ASTFRRGAAQ	GLRGARASAA	ADTLSMRTSA
	60	70	80	90	100
	RAAPRHQQQA	RRGGRFPSLV	VCASAGMNVV	FVGAEMAPWS	KTGGLGDVLG
	110	120	130	140	150
	GLPPAMAANG	HRVMVVSPLY	DQYKDAWDS	VVSEIKMGDG	YETVRFFHCY
10	160	170	180	190	200
	KRGVDRVFVD	HPLFLERVWG	KTEEKIYGPV	AGTDYRDNQL	RFSLLCQAAL
	210	220	230	240	250
	EAPRILSLNN	NPYFSGPYGE	DVVFVCNDWH	TGPLSCYLKS	NYQSHGIYRD
	260	270	280	290	300
15	AKTAFCIHNI	SYQGRFAFSD	YPELNLPERF	KSSFDFIDGY	EKPVEGRKIN
	310	320	330	340	350
	WMKAGILEAD	RVLTVSPYYA	EELISGIARG	CELDNIMRLT	GITGIVNGMD
	360	370	380	390	400
	VSEWDPSRDK	YIAVKYDVST	AVEAKALNKE	ALQAEVGLPV	DRNIPLVAFI
20	410	420	430	440	450
	GRLEEQKQPD	VMAAAIPQLM	EMVEDVQIVL	LGTGKKKFER	MLMSAEKFP
	460	470	480	490	500
	GKVRVAVKFN	AALAHHIMAG	ADVLAVTSRF	EPCGLIQLQG	MRYGTPCACA
	510	520	530	540	550
25	STGGLVDTHI	EGKTGFHMGR	LSVDCNVVEP	ADVKKVATTL	QRAIKVVGTP
	560	570	580	590	600
	AYEEMVRNCM	IQDLSWKGPA	KNWENVLLSL	GVAGGEPGVE	GEEIAPLAKE
NVAAP					
30					
SEQ ID NO: 2 Sugary 2 Protein in maize					
	10	20	30	40	50
35	MSSAAVSSSS	STFFLALASA	SPGGRRRARV	GSSPFHTGAS	LSFAFWAPPS
	60	70	80	90	100
	PPRAPRDAAL	VRAEAEAGGK	DAPPERSGDA	ARLPRARRNA	VSKRRDPLQP
	110	120	130	140	150
	VGRYGSATGN	TARTGAASCQ	NAALADVEIK	SIVAAPPTSI	VKFPAPGYRM
	160	170	180	190	200
40	ILPSGDIAPE	TVLPAPKPLH	ESPAVDGDSN	GIAPPTVEPL	VQEATWDFKK
	210	220	230	240	250
	YIGFDEPDEA	KDDSRVGADD	AGSFEHYGDN	DSGPLAGENV	MNVIVVAAEC
	260	270	280	290	300
	SPWCKTGGLG	DVVGALPKAL	ARRGHRVMV	VPRYGDYVEA	FDMGIRKYYK
45	310	320	330	340	350
	AAGPVNYFHA	FIDGVDFVFI	DAPLFRHRQD	DIYGGSRQEI	MKRMILFCKV
	360	370	380	390	400
	AVEVPWHVPC	GGVCYGDGNL	VFIANDWHTA	LLPVYLKAYY	RDHGLMQYTR
	410	420	430	440	450
50	SVLVIHNIAH	QGRGPVDEFP	YMDLPEHYLQ	HFELYDPVGG	EHANIFAAGL
	460	470	480	490	500
	KMADRVTVS	RGYLWELKTV	EGGWGLHDII	RSNDWKINGI	VNGIDHQEWN
	510	520	530	540	550
	PKVDVHLRSD	GYTNYSLETL	DAGKRQCKAA	LQRELGLEVR	DDVPLLGFIG
55	560	570	580	590	600

5 RLDGQKGVDI IGDAMPWIAG QDVQLVMLGT GRADLERMLQ HLEREHPNKV
610 620 630 640 650
RGWVGFSVPM AHRITAGADV LVMPSTRFEPCLNQLYAMAY GTVPVVHAVG
660 670 680 690 700
GLRDTVAPFD PFSDAGLGWT FDRAEANKLI EALRHCLDTY RNYEESWKSL
710 720
QARGMSQDLS WDHA AE LYED VLVKAKYQW

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Hongxin		JIANG		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Bridgewater	State/Province	NJ	Country of Residence	US
Mailing Address of Inventor:					
Address 1	10 Finderne Ave, Bridgewater, NJ 08807 USA				
Address 2					
City	Bridgewater	State/Province	NJ		
Postal Code	08807	Country	US		
Inventor 2					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Brad		OSTRANDER		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Zionsville	State/Province	IN	Country of Residence	US
Mailing Address of Inventor:					
Address 1	5521 W. 74th St.				
Address 2					
City	Indianapolis	State/Province	IN		
Postal Code	46268	Country	US		
Inventor 3					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chris		LANE		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US		
		Application Number			
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY				
City	Bridgewater	State/Province	NJ	Country of Residence ⁱ	US
Mailing Address of Inventor:					
Address 1	10 Finderne Ave, Bridgewater, NJ 08807 USA				
Address 2					
City	Bridgewater	State/Province	NJ		
Postal Code	08807	Country ⁱ	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence information of this application.			
Customer Number	81552		
Email Address	ZPATDCDOCKETING@COOLEY.COM	Add Email	Remove Email

Application Information:

Title of the Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		
Attorney Docket Number	INGR-036/01US	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	7	Suggested Figure for Publication (if any)	1

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	81552		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	15090926	2016-04-05
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Remove
			Access Code ⁱ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐	This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.
--------------------------	---

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☒ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☒ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US
		Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant 1			
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
Clear			
☐ Assignee	☐ Legal Representative under 35 U.S.C. 117	☐ Joint Inventor	
☒ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
Name of the Deceased or Legally Incapacitated Inventor:			
If the Applicant is an Organization check here. ☒			
Organization Name	Corn Products Development, Inc.		
Mailing Address Information For Applicant:			
Address 1	Av. Do Cafe, 277 Torre B, 2 andar, Jabaquara		
Address 2			
City	Sao Paulo	State/Province	
Country	BR	Postal Code	04311-000SP
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button.			

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	INGR-036/01US	
		Application Number		
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY			

Assignee 1				
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.				

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the **INITIAL** filing of the application and either box A or B is **not** checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Jonathan M.Cousin/			Date (YYYY-MM-DD)	2016-04-21
First Name	Jonathan	Last Name	Cousin	Registration Number	66418
Additional Signature may be generated within this form by selecting the Add button.					

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	INGR-036/01US
	Application Number	
Title of Invention	COMPOSITIONS AND METHODS FOR PRODUCING STARCH WITH NOVEL FUNCTIONALITY	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.