Title

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Authors

Beckles, DM

Thitisaksakul, M

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Use of Biotechnology to Engineer Starch in Cereals

Diane M. Beckles and Maysaya Thitisaksakul
Department of Plant Sciences, University of California—Davis, Davis, California, U.S.A.

Abstract
The starch accumulated in rice, wheat, maize, sorghum, and millet grain is indispensible for human survival as it accounts for most of the consumed calories. In addition, cereal starch is used in its natural or modified state as a healthful food, and as an environmentally friendly additive or replacement for petroleum-derived fuel and polymers. Therefore, developing cereals that accumulate higher endosperm starch and starches with novel polymeric properties could help to meet the dual challenges of sustaining human population growth needs while minimizing some of the harmful environmental impacts. Despite its fundamental importance, comparatively little is known about the mechanistic basis of starch biosynthesis. This entry provides a basic overview of the "starch field" for the beginner. First, the various uses of starch, its structural organization and biosynthesis, and how its functionality relates to its structure are outlined. Second, how recent biotechnological advancements are leading to the discovery of new genes that modulate starch and to novel starches generated through genetic engineering are described. Finally, some of the remaining questions and challenges that must be tackled in order to meet the goal of increasing starch production for use as a food, feed, fiber, and polymer in the next 50 years and beyond are illustrated.

INTRODUCTION

Starch is a natural polymer of glucose. It is deposited as water-insoluble granules in most plant tissues, but in cereal it makes up ~70–90% of endosperm dry weight, and serves as a dense and rich source of carbon and energy.[1] Depending on the nutritional status of the plant, starch biosynthesis can be triggered, enhanced, slowed down, or even inhibited to suit plant growth requirements. The judicious allocation of carbon from photosynthetic tissues to the seeds for long-term storage enhances survivability in the next generation and thereby increases plant fitness.[2]

Storage starch also plays a critical role in the human diet, accounting for 50–80% of all calories consumed. In many economically advanced countries, cereal starch is abundant and cheap and as a result consumers expect starch products to provide not only basic nutrition but also healthful benefits and high hedonistic value. For example, in many Asian markets, few compromises can be made with respect to the sensorial property of rice starch where texture and translucency are critical, and in North America and Europe, there is a surge of interest in slow-release, low-digestible starches.[3] These starches behave in a physiologically similar way to fiber by creating an enhanced prebiotic effect that is associated with reduction in the occurrence of colon cancer.[4]

The abundance, low cost, and physico-chemical versatility of starch also makes it competitive as a raw material for the large-scale biomaterial processing sector.[5] About 35% of all starch in Western countries is used in its native or modified form as biopolymers in the food, textile, and paper manufacturing industries (Fig. 1). Maize starch use in the United States exemplifies this; most of it is not directly consumed, but is converted into a diverse set of products, ranging from animal feeds to sweeteners, to polymers, and fuels.[3] Many value-added starch-based biopolymers and starch-derived biofuels are projected to be less harmful to the environment than those derived from petrochemicals.[5] What these examples illustrate is that there is a need for cereals producing a wide array of starches for non-food uses, and also, for increased starch production to ensure global food security. The goal of this entry, therefore, is to examine the many attempts to engineer starch to optimally meet its growing and diverse end-uses.

ORGANIZATION OF THE STARCH GRANULE

Starch is composed of two large glucose polymers called amylose and amylopectin. Amylose is smaller, comprising 20–30% of the dry weight of normal starch, while amylopectin may be up to 100 times larger than amylose and makes up 60–70% of starch. Both polymers consist of glucose molecules connected by α-1-4-linkages creating glucan chains which are occasionally branched by α-1-6-linkages. The frequency of branching is higher in amylopectin (Fig. 2),[1] and is highly ordered so that chains of 3–4 signature lengths are created. These chains are arrayed to form clusters interspersed by regions with branch points (Fig. 2C).[2] The specific arrangement and architecture of these glucan chains permits their molecular self-assembly into a semicrystalline macromolecule and eventually to granules of distinct sizes and morphologies at maturity (Fig. 2).[1] The morphology, sizes, and relative numbers of the granules

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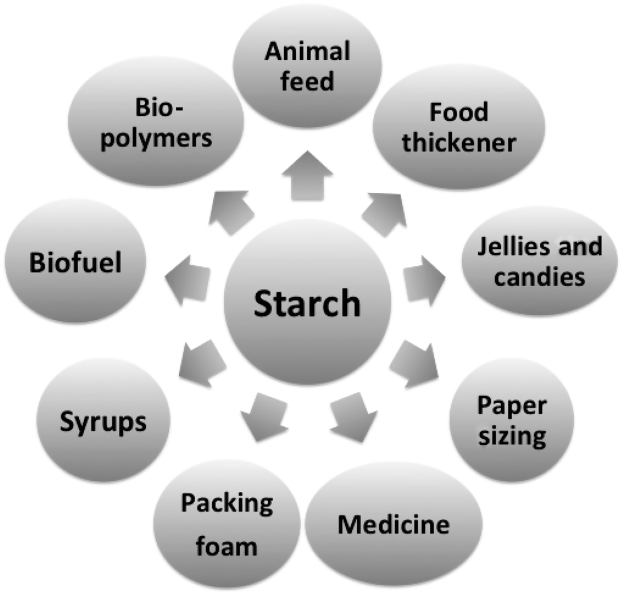

Fig. 1 Non-food uses of starch. Native starches may be modified chemically, physically, enzymatically, or by a combination of these processes to create a material that is specifically designed to one of these uses.

in the endosperm are often representative of the species from which the starch was isolated. Granule shapes in cereal starches vary from lenticular, polyhedral, spherical, to oval. They can be single or compounded, range from 1 to 150 μm, and have a unimodal or bimodal distribution of size.[1]

HOW IS STARCH MADE?

Sucrose produced from photosynthesis is imported into the endosperm and is metabolized to hexose phosphates (Fig. 3). The hexose phosphate is combined with adenosine triphosphate (ATP) to synthesize adenosine diphosphate (ADP)glucose, a building block for the biosynthesis of the glucan chain (Fig. 3). Starch synthases (SSs) use the glucosyl moiety of ADPglucose to create α-1,4-linked chains of distinct lengths. Starch branching enzymes (SBEs) and starch debranching enzymes (DBEs) together determine the branching pattern of starch, which is key to maintaining amylopectin crystallinity. Starch phosphorylase (PHO) releases hexose phosphate from starch, which are reused as substrates for amylopectin synthesis (Fig. 3).[2] Amylose is synthesized by a single enzyme granule-bound starch synthase (GBSS), while amylopectin is synthesized by the synergistic action of multiple isoforms of SSs, SBEs, and DBEs.

Multiple isoforms of each of these enzymes exist, each with varying specificities or spatio-temporal occurrences (Fig. 3). For example, each SS and SBE isoform uses glucan chains of specific length as a substrate, and the products of some enzymes act as the substrate of others. Therefore, the collection of starch biosynthetic isoforms in a cell at a given time, developmental stage, or genotype may define the physico-chemical signature of the starch granule made and may partially explain the differing granule characteristics.[2] The spectrum of starches available for modification could be significantly increased if plants contain an even greater variation in the combination of different starch biosynthetic isoforms through biotechnological means.[2]

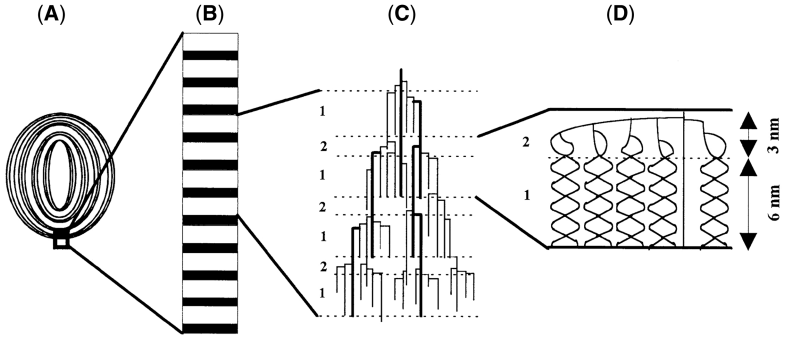

Fig. 2 Hierarchical organization of the starch granule. (A) Internal growth ring structure of a starch granule. These may arise in some species due to diurnal variation in leaf photosynthate substrate supply to the endosperm, which is high during the day and reduced at night. Regions high in crystallinity alternate with those that are more amorphous. (B) Organization of the semicrystalline growth ring. Layers of alternating amorphous (black) and crystalline lamellae (white) are visible. (C) Arrangement of glucan chains in the semicrystalline growth ring. The α-1,4-glucan linear chains are arrayed in the crystalline regions (labeled 1), while the α-1,6-branched points are aligned so that they form the amorphous region of the granule (labeled 2). The α-1,4-glucan chains are classified as A-, B-, and C-chains according to the number of glucoses they contain with A-chains being the shortest and C-chains the longest. A-chains are only attached to a single chain, while B-chains may span 1, 2, or 3 clusters (shown as bold lines). There is a single C-chain, which has the reducing glucose molecule (not shown). (D). One unit of crystalline/amorphous lamellae is 9 nm in width and is constant in all starches. Adjacent chains (consisting of at least 10 glucose units) can form double helices. In this example, the 6 nm crystalline region consists of ~18 glucoses. In cereals, these helices are arranged into A-type crystallites. The arrangement of amylose is not shown here but may be localized to the amorphous region of the amylopectin structure.

Source: Reprinted from Ball, Guan, et al.[6] with permission from Elsevier.

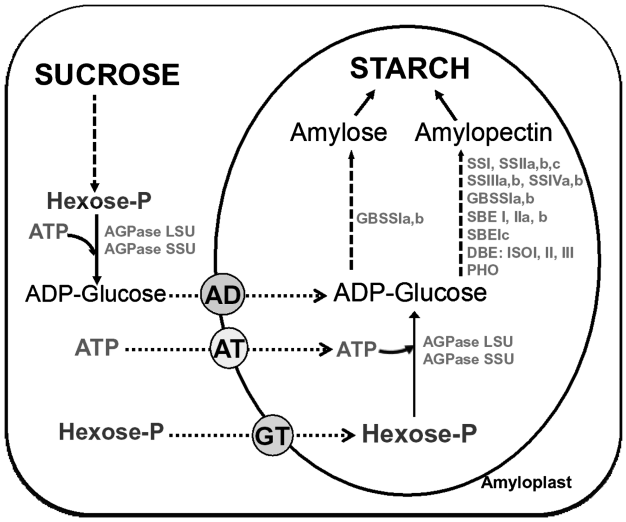

Fig. 3 Starch biosynthetic pathway. Sucrose is imported into the endosperm cell and is metabolized to hexose phosphates, often by sucrose synthase (SuSy; not shown). These hexose phosphates serve as substrates for starch, protein, and oil biosynthesis. In developing endosperm, most hexose phosphate is used for starch biosynthesis. ATP generated from oxidative phosphorylation and glycolysis is also needed to drive these energy-intensive reactions. Enzyme or transporter activities that affect ATP and hexose phosphate availability upstream of the synthesis of ADPglucose can influence the amount and molecular architecture of the starch made. AGPase: ADPglucose pyrophosphorylase; AD: ATP/ADPglucose transporter; AT: plastidial ATP transporter; GT: glucose-6-phosphate transporter; SS: starch synthase; GBSSI: granule-bound starch synthase I; SBE: starch branching enzyme; DBE: starch debranching enzyme; PHO: starch phosphorylase. The various isoforms of these genes that have been cloned, e.g., SSI, II, III, and IV, are shown; however, a functional role in cereal endosperm starch synthesis has not been directly determined for SSIV, SSIIc, SBEIII, and SBEIc.
Source: Adapted from Thitisaksakul, Jimenez, et al.[1]

HOW IS THE STRUCTURE OF STARCH RELATED TO ITS FUNCTION?

Starches of different molecular structures are exploited to create products with a broad continuum of functionalities (Fig. 1). The proportion of amylose to amylopectin, the amount of lipid complexed with amylose, the ratio of A-to-B glucan chains in amylopectin, glucan chain helical conformation, and granule size and morphology collectively dictate starch properties. They do so by altering starch gelatinization, viscosity, swelling power, and retrogradation.[3]

The amylose-to-amylopectin ratio is often a key target for starch improvement. The null mutation in *GBSSI* (*waxy*) results in the loss of amylose production in cereal starches, while the loss in SBEs activity could lead to a starch with high amylose content. Amylose content is negatively correlated with granule sizes. As a result of having little to no amylose, waxy starch is more susceptible to digestion than normal and high-amylose starch (Fig. 4). It also requires less time and lower temperatures to become gelatinized, producing a clear paste, which remains viscous over time. Waxy starch is, therefore, ideal for use as a food thickener and as an adhesive in many industries. On the contrary, high-amylose starches, which are resistant to digestion, may serve as a source of dietary fiber, providing low-glycemic-index carbohydrates for individuals who require strict glycemic management (Fig. 4).[5] These examples illustrate how genetic alterations in starch enzymes can profoundly influence starch end-use.[3] This spectrum of starch uses is further extended by modifying native starches enzymatically or chemically, e.g., by acetylation, phosphorylation, acidification, methylation, and propylation, and by subjecting it to physical treatments, such as spray drying or extrudation of gelatinized starch.[5]

BIOTECHNOLOGICAL MANIPULATION OF STARCH

The primary target for engineering starch is to increase the amount produced per unit area of land and to maintain yield stability under stress. Starches with unique properties are much sought after as the uses of starch are as varied as its molecular structure (Figs. 1 and 4). There are two broad methodologies for manipulating plants through biotechnological means: reverse genetics, which are targeted approaches, and forward genetics, which are open-end approaches (Fig. 5). These are discussed below.

Targeted Approaches (Reverse Genetics)

Gene sequences that may encode starch metabolic enzymes can be identified using bioinformatics and their functions tested by reverse genetics methods such as transgenic modifications and Targeting Induced Local Lesions IN Genomes (TILLING). In discovering the functions of these candidate genes, unique starches may be produced that can be translated into a commercial pipeline for starch improvement.

Transgenic Modification

This method changes the genetic makeup of an individual by introducing deoxyribonucleic acid (DNA) into its genome so that it expresses: i) a foreign gene; ii) a native gene that was modified; iii) a native gene at decreased or increased levels; or iv) any combination of i) to iii). Over the years, several of the individual starch gene isoforms have been altered using potato as a model because of the high transformation efficiency, short generation time, and large amount of starch made in the tuber. However, the efficiency of cereal transformation has improved, and the roles of many genes in cereal starch biosynthesis are being tested directly. For example, high-amylose bread and pasta wheat,

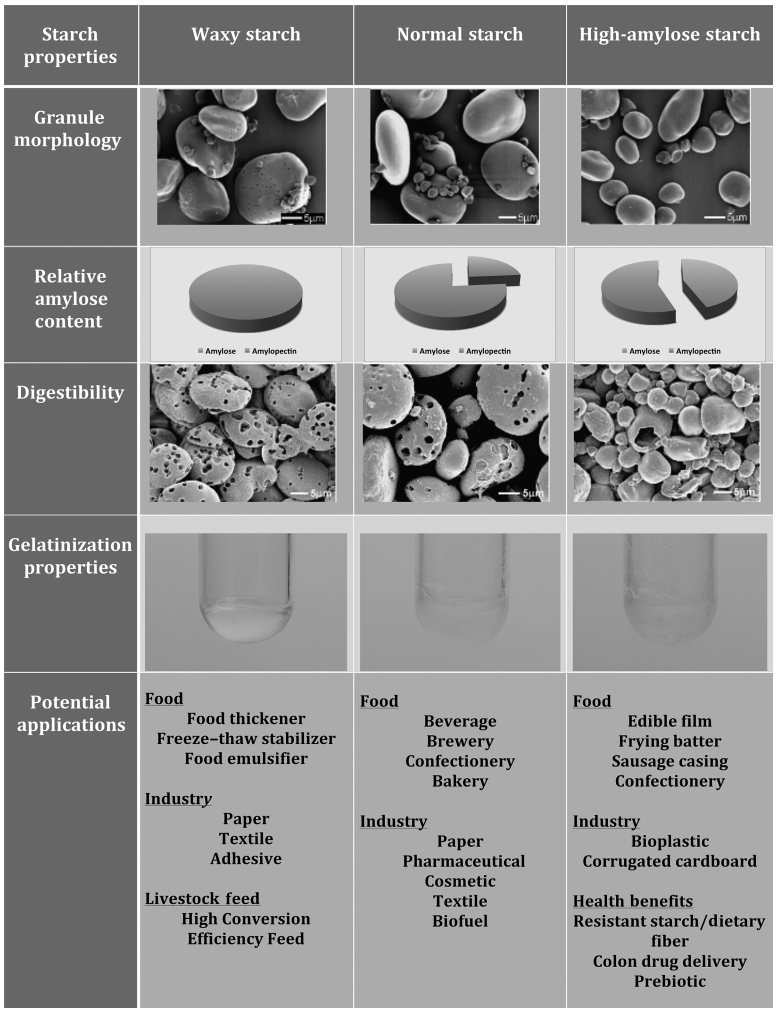

Fig. 4 The relationship between changes in starch biosynthetic enzyme activity and its influence on starch composition, granule properties, functionality, and applications. Waxy, normal, and resistant starches have low, normal, and high amylose content, respectively. The difference in amylose content leads to variation in starch granule morphology, digestibility, pasting properties, and industrial usages. The starch (20 mg) in the figure was gelatinized by boiling for 5 min. It was isolated from waxy (CDC Alamo), normal (CDC Dawn), and high-amylose (SB 94893) barley seeds, which were a kind gift from Dr. Aaron Beattie.
Source: Scanning electron micrographs of native starch and starch digested with porcine pancreatic α-amylase were reprinted from Li, Vasanthan, et al.[7,8] with permission from Elsevier.

barley, and rice genotypes were achieved by the transgenic knockdown of *SBEI*, *SBEIIa*, and *SBEIIb* using RNA interference (RNAi) technology. Sequential and combinatorial starch metabolic gene knockdowns and knock-ups have been implemented to produce starches with a wide array of novel properties.[4] Simultaneous reductions of SBEI, SBEIIa, and SBEIIb activity in barley led to starch with 100% amylose.[9] While producing this all-amylose starch

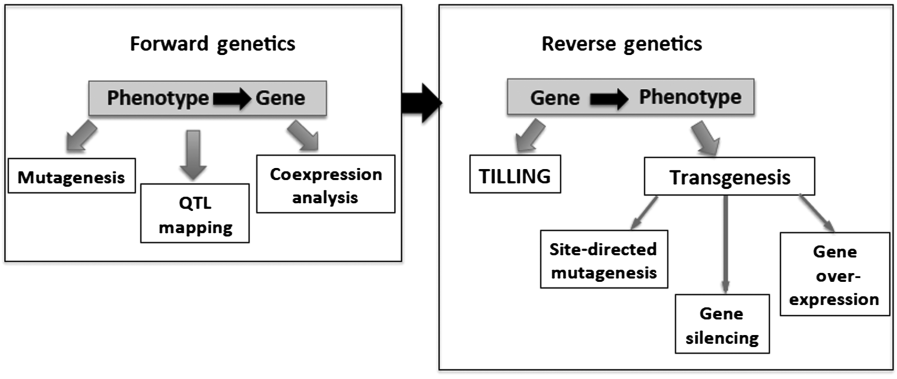

Fig. 5 Biotechnological approaches to altering the genes that may control starch biosynthesis. These may be broadly defined as either forward or reverse genetics approach. The forward genetics approach uses variation in the trait among different individuals and then seeks to identify the causal gene. The reverse genetics approach starts with a candidate gene of unknown function but based on indicative sequence motifs or domains present it may be predicted to be involved in a particular process(es), which is then tested. Often, candidate genes identified by forward genetics are verified using reverse genetics. Further descriptions of the different experiments are given in the entry.

was relatively straightforward; rarely is it possible to predict the nature of the starch that will result from multienzyme engineering. This is because of the highly interactive manner in which the different starch biosynthetic enzymes operate to make starch.[2]

Increasing the substrate available for import into the plastid, and its subsequent utilization by the amylose and amylopectin biosynthetic enzymes therein could enhance cereal yields (Fig. 3). This has been accomplished by increasing sucrose synthase (SuSy) activity in maize and ADPglucose pyrophosphorylase (AGPase) activity in wheat and rice.[10] AGPase was engineered by site-directed mutagenesis so that the enzyme had altered allosteric regulatory sites and increased activity. In potato tubers, higher plastidial ATP/ADP transporter (AD), plastidial adenylate kinase, and SSIV activity also increased starch content.[10] To our knowledge, the functional roles of these genes in starch biosynthesis have not yet been determined in cereals.

TILLING

This is a high-throughput Polymerase Chain Reaction (PCR)-based method of screening a mutant collection (randomly generated using chemical or physical mutagenic agents), for alterations in the sequence of a gene of interest. Depending on the location of the mutation, the gene product may be defunct, reduced in, or altered in activity. These novel alleles can be integrated into cereal germplasm by backcrossing the mutants with a commercial line to alter starch composition. TILLING populations have been developed for rice, wheat, barley, sorghum, and oat. This method is becoming popular because it is a non-transgenic alternative to genetic engineering as crops produced in this way are easier to commercialize. High-amylose cereals with high "apparent fiber" are one of the most desired phenotypes created using this method. Using TILLING to change the nutritional properties of wheat is of particular interest because it accounts for 20% of human caloric intake, but wheat products produced by transgenesis are not accepted in the marketplace. Durum and bread wheat with novel alleles of *GBSSI*, and of *SBEIIa*, and *SBEIIb* were developed by TILLING; establishing waxy and resistant starch phenotypes, respectively.[4]

Open-Ended Approaches (Forward Genetics)

The forward genetics approach toward manipulating starch biosynthesis would start by connecting an observed variation in a starch trait among a group of related organisms to an underlying gene(s) or alleles of that gene. This approach assumes that our knowledge of a biological process is incomplete and that there are potentially new alleles, genes, and gene networks that control the phenotype. There are several forward genetics approaches but only three are outlined here: i) mutagenesis; ii) quantitative trait loci (QTL) mapping; and iii) coexpression analysis. Once identified, the causal genes or alleles of the genes can be integrated into the commercially important cereal varieties through breeding or TILLING, thus establishing a cereal genotype with desirable starch traits.

Mutagenesis

A large population of seeds is exposed to a DNA-altering agent, which may be chemical or physical, with the hope of mutagenizing a gene(s) that affects starch accumulation or structure. If successful, the mutation would be manifested as a seed with aberrant starch. Gross changes to the starch biosynthetic pathway can be easily screened (Table 1). For example, seeds accumulating higher starch would be larger, those containing starch with significant shifts in the amylose-to-amylopectin ratio would stain brown rather than the characteristic blue-black color with iodine, and mutations reducing starch biosynthesis would lead to wrinkled or shrunken seeds after harvest.[5] Many starch biosynthetic genes in maize were discovered by analyzing seeds with a "shrunken" and "brittle" kernel appearance. Using molecular and genetic analysis, the causal genes were identified as i) the small and large subunit of AGPase (*bt2* and *sh2*, respectively); ii) sucrose synthase (*sh1*); and iii) the ADPglucose transporter (*bt1*) among others.

QTL Analysis

Cereal species and genotypes that are not used commercially often possess starches with unique structural and morphological diversity and can often maintain starch production under environmental stress. The alleles underlying these valuable traits may be uncovered by QTL mapping, either through map-based positional cloning, or by association studies. If there is a statistical connection or co-occurrence of a starch characteristic and a DNA sequence polymorphism among a collection of hundreds of related species, then that allelic variant may be causal for the trait.

Analysis of Segregating Populations. The F_2 population derived from a cross of two parents with stark contrasts in starch functionality can be useful for finding regions of the genomes, i.e. QTLs, that contribute to those traits. With a concerted effort, the genes may be positionally cloned. QTL studies have been done to map genes for starch functionality in popcorn and sweet corn, amylose, amylopectin, and starch content in maize,[11] chalkiness and eating quality in rice,[12] pasting properties in barley,[13] and granule size in wheat species[14] among others. Recently, it was discovered that the effects of some mutant starch alleles on functionality may vary depending on the genotype in which the mutation was introduced.[15] Therefore, the unique metabolic and physiological state of the cell may strongly affect the starch made. This possibility is being further explored by using MAGIC (multiparent

Table 1 Effect of mutations in grain starch accumulation and functionality in the major isoforms of cereal endosperm starch biosynthetic enzymes

Gene	Species	Mutant	Change in starch		Reference
			Content	Functionality	
AGPSSU	Rice	*osagps2*	Yes	N/A	Lee et al. (2007)
	Barley	*riso¸16*	Yes	A-granule misshaped	Johnson et al. (2003)
	Maize	*brittle2 (bt2)*	Yes	N/A	Tsai and Nelson (1966)
AGPLSU	Rice	*osagpl2*	Yes	N/A	Lee et al. (2007)
	Maize	*shrunken2 (sh2)*	Yes	N/A	Tsai and Nelson (1966)
GBSS	Rice	*waxy*	N/A	Low amylose	Sano (1984)
	Barley	*waxy*	N/A	Low amylose	Patron et al. (2002)
	Maize	*waxy*	N/A	Low amylose	Tsai (1974)
	Wheat	*waxy*	N/A	Low amylose More long chain (DP > 19)	Nakamura et al. (1995) Fujita et al. (2001)
SSI	Rice	*ssI*	No	Yes	Fujita et al. (2006)
SSIIa	Rice	*alk* (most Japonica)	N/A	Yes	Umemoto et al. (2001)
	Barley	*sex6*	N/A	High amylose	Morell et al. (2003)
	Maize	*sugary2 (su2)*	N/A	Yes	Zhang et al. (2004)
	Wheat	*sgp1*	Yes	Yes	Yamamori et al. (2000)
SSIIIa	Rice	*flo5*	No	Yes	Fujita et al. (2007)
	Maize	*dull1*	N/A	N/A	Gao et al. (1998)
SBEI	Rice	*starch-branching enzyme1 (sbe1) flo2*	No	Yes Yes	Satoh et al. (2003) Kawasaki et al. (1996)
SBEIIa	Rice	*beIIa*	N/A	No	Nakamura (2002)
SBEIIb	Rice	*amylose extender (ae)*	N/A	High amylose	Nishi et al. (2001)
	Maize	*amylose extender (ae)*	N/A	High amylose	Stinard et al. (1993)
ISA	Rice	*sugary1(su1)*	N/A	Phytoglycogen	Kubo et al. (1999)
	Barley	*riso 17, notch2*	Yes	Granule number and form	Burton et al. (2002)
	Maize	*sugary1 (su1)*	N/A	Granule number and form	James et al. (1995)
PUL	Rice	*pul*	N/A	Slight difference	Fujita et al. (2009)
	Maize	*zpu1*	N/A	N/A	Dinges et al. (2003)
Pho1	Rice	*pho1*	Yes	Smaller granules, altered amylopectin	Satoh et al. (2008)

Source: Adapted from Jeon, Ryoo, et al.[17] with modifications.

advanced generation intercross) wheat lines. The genomes of 4–8 parents (or more) are combined through multiple and sequential crossings so that the developed lines have a mosaic of genomes from diverse parents. The expectation is that the novel allelic interactions which may occur among starch pathway intermediates in the new lines can lead to new types of starches.[4]

Association Studies. Genome-wide association studies (GWAS) are the most powerful techniques for gene discovery and rely on having a complete or near-complete genome sequence of multiple individuals available. Using robust statistical analysis, connections can be made between variation in starch and an allele-type(s) among the population. GWAS could lead to the discovery of new, hitherto unknown genes that can influence starch accumulation. Such an attempt was made in barley, which was screened for candidate genes linked to resistant starch. A variety of genes were implicated, including SSIV.[16]

A more conservative method that is widely used is identifying sequence polymorphisms or differences in select starch metabolic genes among individuals in a population that differ in endosperm starch. In rice, sequence polymorphisms in *GBSSIb* and *SSIIa* were associated with variation in amylose content and rice cooking quality. In sorghum, polymorphisms in *SSIIa* and *SBEIIb* were linked to variation in starch gelatinization temperature, while polymorphisms in *GBSSI* were related to variation in starch

Table 2 Identified transcriptional regulators of starch biosynthesis and structure in cereals

Species	Gene	TF family	Gene targets	Phenotype	Reference
Barley	*SUSIBA 2*	WRKY	*SBEIIb, ISO1*	Negative regulator	Sun et al. (2003)
Rice	*OsBP-5* *OsEBP-89*	MYC protein EREBP protein	*GBSSI*	Positive regulator of amylose	Zhu et al. (2003)
Rice	*OsbZIP58*	b-ZIP	*OsAGPL3, Wx, OsSSIIa, SBE1, OsBEIIb*, and *ISA2*	Positive regulator. Reductions leads to lower starch, amylose, and the proportion of short and intermediate chains of amylopectin	Wang et al. (2013)
Rice	*FLO2*[a]	Uncharacterized protein that mediates protein–protein interaction	*bHLH, LEA*	Positive regulator. Reductions leads to lower amylose, higher proportion of intermediate length chains and lower short and long chains	She et al. (2010)
Rice	*RSR1*	AP2/EREBP	*SBEI, SBEIIb, SSI, SSIIa,* and *SSIIIa*	Negative regulator. Reductions increase starch and amylose, alters granule morphology and size and changes functionality	Fu et al. (2010)
Rice	*SERF1*	SALT-RESPONSIVE ERF1	*RPBF, GBSSI*	Negative regulator of RPBF which in turn modulates starch	Schimdt et al. (2014)
Rice	*RPBF*	PROLAMIN-BOX BINDING FACTOR		Positively regulates starch content	Kawakatsu et al. (2009)

[a]Not discovered by coexpression analysis.
Source: Adapted from Schmidt, Schippers, et al.[18]

accumulation. In barley, sequence variation in *SuSyI* and *II* correlated with higher starch after drought stress, and in maize, selection for cooking quality during domestication was associated with polymorphisms in *SBEIIb* and *GBSSI*.[1,4]

Coexpression Analysis

Publicly available metadata sets of gene expression studies in cereal endosperm can be mined to find novel genes that are synchronously expressed with starch amount or with known starch enzymes. Regulatory genes expressed in concert with starch genes may be primary candidates for "starch supraregulators." Several genes have been so identified and in some cases, genetically altering the amount of a regulator caused changes in starch amount and structure (Table 2).

CHALLENGES/FUTURE DIRECTIONS

Despite many attempts to uncover the players and regulators of the starch biosynthesis pathway, the picture of how this glucose polymer is biosynthesized is still fragmentary. How granules are initiated, what determines the placement of the branches and glucan chain length, what determines granule size and the amylopectin structure, and how the different proteins interact with each other to build starch are still largely unknown. Even target genes that are predicted to increase starch levels, when modified, rarely give expected results. Below are some challenges and potential future directions.

1. A basic understanding of the regulation of starch biosynthetic activities at the transcriptional and posttranscriptional level is needed. Being able to dissect these regulatory mechanisms would enable scientists to enhance the plants' ability to produce a starch polymer that would suit the use of both agricultural and industrial sectors without significantly changing the amount accumulated.
2. The nanostructural arrangement of the starch molecule still remains elusive. Filling in the gap in our understanding of starch molecular architecture may be helpful in predicting its downstream product quality, and would facilitate more precise tailoring of starch.
3. Starch biosynthesis is heavily modulated by the environment. Many starch traits may have low heritability, which makes developing new germplasm challenging. In addition, starch quality and accumulation varies considerably depending on environmental conditions and this will become more problematic as weather patterns become more extreme. It would be desirable to understand how the starch enzymes are regulated by environmental stress and their role in hormonal signal transduction pathways.
4. Many starch enzymes are physically clustered together and work cooperatively to build the starch granule. The ability to ascribe a precise function to each enzyme

isoform in making starch may only be possible if the role of these complexes is better understood.

5. Engineering starch enzymes to have new binding and kinetic capacities rather than a gross change in levels may lead to "unconventional" starches without drastic reductions in the amount made. This was demonstrated with AGPase, which was engineered to have altered allosteric regulatory sites. These changes either directly or pleiotropically increased overall plant yield.

CONCLUSION

Starch is one of the most important products made by plants. More attention needs to be focused on understanding the regulatory mechanisms that controls its biosynthesis. This is a basic requirement in any effort to sustain current and future human population growth and accommodate the widening use of starch as a biomaterial and biofuel. With the current understanding of enzymes and regulatory proteins involved in the starch biosynthesis process, and with the development of many state-of-the-art biotechnological techniques, several cereal varieties harboring desirable starch properties and yield have been generated via biotechnological means, and in the next few years we are poised to make significant inroads by building upon genetic and genomic resources and rapid advances in starch analytical techniques.

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