

Different genetic strategies to generate high amylose starch mutants by engineering the starch biosynthetic pathways

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ABSTRACT

This review systematically documents the major different strategies of generating high-amylose (HAS) starch mutants aiming at providing high resistant starch, by engineering the starch biosynthesis metabolic pathways. We identify three main strategies based on a new representation of the starch structure: 'the building block backbone model': i) suppression of starch synthases for reduction of amylopectin (AP) side-chains; ii) suppression of starch branching enzymes (SBEs) for production of AM-like materials; and iii) suppression of debranching enzymes to restrain the transformation from over-branched pre-AP to more ordered AP. From a biosynthetic perspective, AM generated through the second strategy can be classified into two types: i) normal AM synthesized mainly by regular expression of granule-bound starch synthases, and ii) modified linear AP chains (AM-like material) synthesized by starch synthases due to the suppression of starch branching enzymes. The application of new breeding technologies, especially CRISPR, in the breeding of HAS crops is also reviewed.

1. Introduction

Starch is one of the most important primary food commodities and industrial feedstocks on the globe. It consists of two main macromolecular entities: the mainly linear α -1,4 linked amylose (AM) and the more branched α -1,4- α -1,6 branch-linked amylopectin (AP). The typical AM content of starch in our main starch crops, such as wheat, rice, rye, potato, maize, and cassava, ranges between 20% and 30%. (Moorthy, Andersson, Eliasson, Santacruz, & Ruales, 2006) Genotypes including the so-called waxy starch (high AP) and high AM starch (HAS) provide an array of different important functionalities.

HAS has two important assets which are related to its relative robustness toward thermal and enzymatic treatments: its usefulness for bio-based materials and high nutritional value based on its high resistant

starch (RS) content. RS is the fraction of the starch that resists the upper human digestive system and is fermented in the large bowel, thereby reducing the glycemic response and providing protection against colorectal cancers and high plasma cholesterol and triglyceride levels (Zhong et al., 2019). The industrial use of HAS includes films, coating, textiles, paper, medical devices, and future biodegradable flexible packaging (Li, Dhital, Flanagan, et al., 2020; Li, Gidley, & Dhital, 2019). The steadily increasing number of diverse HASs provides a unique potential for different applications to solve escalating health and sustainability-associated problems.

A number of reviews on HAS deal with its fundamental nutritional value as RS, especially methods of preparation, quantification, and health-promoting effects (Perera, Meda, & Tyler, 2010; Sajilata, Singhal, & Kulkarni, 2006). Specifically, the analytical methods for determining

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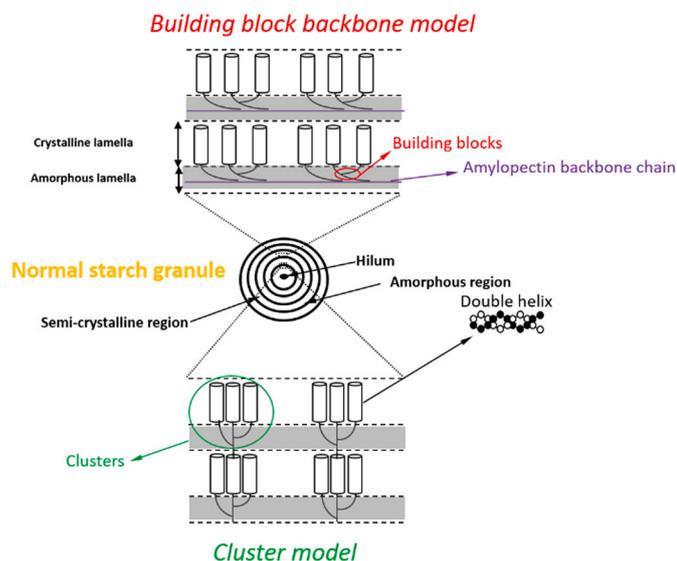


Fig. 1. The classical 'cluster model' and the 'building block backbone model' of AP in a starch granule lamellar segment. Cylinders denote double-helical segments.

RS and the effect of starch composition and processing methods on variations in RS contents of pulses and cereal starches have been evaluated (Perera et al., 2010; Sajilata et al., 2006). Starch, particularly the AM fraction, is an exceptional resource for novel materials and, as such, these uses have been reviewed, such as starch-based plastics (Ammala et al., 2011; Chen & Yan, 2020; Shah, Hasan, Hameed, & Ahmed, 2008). The specific effect of AM on the mechanical properties of normal starch-based plastics (Follain, Joly, Dole, & Bliard, 2005), and properties of HAS-based films have been reviewed as well (Dureja, Khatak, Khatak, & Kalra, 2011). A recent review describes HAS in respect to its structure-nutritional functionality relationship with RS (Li, Gidley, & Dhital, 2019). This review is mainly devoted to the application of HAS in functional foods and bioplastics, and how structural features contribute to digestive enzyme resistance of native HAS and food processed HAS.

As AM and HAS are gaining increasing attention, this review addresses relationships between the collected relational data of biosynthesis, structure, new breeding techniques and functionality of HAS to provide a comprehensive report to assist its further development and application. Hence, this review seeks to synthesize new HAS breeding technologies resulting in alterations of the HAS molecular structure, translating into diverse multistructural and nutritional effects, and associate the derived informations based on the new 'building block backbone model'. An understanding of the updated breeding techniques and strategies on generating HAS crops based on the new type of starch structure model is expected to be beneficial for developing new breeding strategies of HAS crops.

2. Structure of HAS

2.1. Overview of starch structure

Native starch granules are multi-scale structured from nanometer to micrometer scales, i.e., AM and AP molecular chains (0.1 nm), crystalline and amorphous lamellar structure (8–11 nm), alternating amorphous and semi-crystalline growth rings (0.1 μm), and starch granules (1–100 μm) (Bertoft, 2017). These granules are semi-crystalline, showing a hierarchical structural periodicity that is apparent at the micro-scale as different concentric layers, with alternating amorphous and semi-crystalline radial growth rings arising from the inner hilum (Blazek & Gilbert, 2011; Kozlov, Blennow, Krivandin, & Yuryev, 2007; Waigh, Perry, Riekel, Gidley, & Donald, 1998).

2.2. Cluster model and building block backbone model

There are two main models describing how AP chains are organized in the branched polymer, namely the "cluster model" and the "building block backbone model" (Bertoft, 2017). The primary difference between the two AP models is, that long AP chains inevitably penetrate the crystalline and amorphous lamellae (cluster model), or the chains are mainly oriented within amorphous lamellae but can also partly transverse the crystalline and amorphous lamellae (building block backbone model) (Fig. 1). The cluster model mainly emphasizes that the short chains in AP are attached together by densely clustered branch points located in the amorphous lamella. Long AP parallel chains interconnect these clusters in a radial manner in the starch granule (Fig. 1) (Nikuni, 1978). This representation has been widely accepted for decades and is based mainly on the clearly polymodal distribution of the AP side chains, intuitively an effect of the supposed clustered chains. However, the accuracy of this model has also been challenged, as new data of the chain structures has become available. Hence to test the validity of the cluster model, the structure of the multiple branched dextrans hydrolyzed by α -amylase was analyzed and partly found to not be consistent with the expected structure of clusters (Bertoft, Koch, & Åman, 2012; Laohaphatanaleart, Piyachomkwan, Sriroth, & Bertoft, 2010). Their main arguments are, that clusters have actually never been identified or isolated and that the cluster model requires a much higher proportion of the very short AP chains, constituting the branched clusters. The revised model termed the "building block backbone model" (Bertoft, 2017) is characterized by three different points: i) The long AP chains are linked to each other and form collectively concentric, not radial, amorphous backbone chain sheets. ii) Branched building blocks formed by 1,6-branch points are attached to the backbone chains and outspread along the amorphous backbone. iii) Short AP chains extend from these branched building block structures and form double helices, which align to form concentric crystalline lamellae (Fig. 1) (Bertoft, 2017). However, the 'cluster model' is also supported by data, e.g., the amyloextrin structure (Kainuma & French, 1971, 1972), the deduction of the left-handed double helix structure from X-ray diffraction pattern data (French, 1972), and transmission electron microscopy images (Yamaguchi, Kainuma, & French, 1979), and the radial orientation of fibrous structure in crushed and fractured potato starch granules (Sterling, 1974). Hence, the rationality of the two models is still under debate.

The general assumptions for the contribution of AM to the lamellar structure are: i) AM co-crystallizes with AP side-chains within crystalline lamellae; ii) AM orients within the amorphous lamellae; and iii) AM tie-chains pass through both the crystalline and amorphous lamellae (Blazek & Gilbert, 2011; Kozlov, Krivandin, Shatalova, Olga, & Bertoft, 2006). This model was recently substantiated by further dividing AM chains into different fractions with different chain lengths, followed by correlation analysis with crystallographic data (Zhong, Liu, Qu, Blennow, et al., 2020). Specifically, the data suggests that i) short AM chains co-crystallize with AP side-chains within the crystalline lamellae; ii) intermediate AM chains penetrate the crystalline and amorphous lamellae as tie-chains; and iii) long AM chains orient within amorphous lamellae (Wu & Gilbert, 2010; Zhong, Liu, Qu, Blennow, et al., 2020). With the limited data available so far, it can only be supposed that the same function of AM is valid for both models, i.e., by forming single and double helices with AM, penetrating the crystalline and amorphous lamellae, oriented within the amorphous lamellae, co-crystallizing with AP side-chains. (Zhong, Liu, Qu, Blennow, et al., 2020). However, it is important to emphasize that the effect of AM on the lamellar structure is also highly dependent on genetic background. Hence, for the wheat starch system with AM content of 1.5%–39.5% (Yuryev et al., 2004), and potato system with AM contents between 2% to 36% (Kozlov et al., 2007), the crystalline lamellar structures were found to be constant irrespective of the AM content. However, positive (Kozhevnikov et al., 2001; Matveev et al., 2001) and negative (Zhong, Liu, Qu, Blennow, et al., 2020) correlations between the thickness of the crystalline

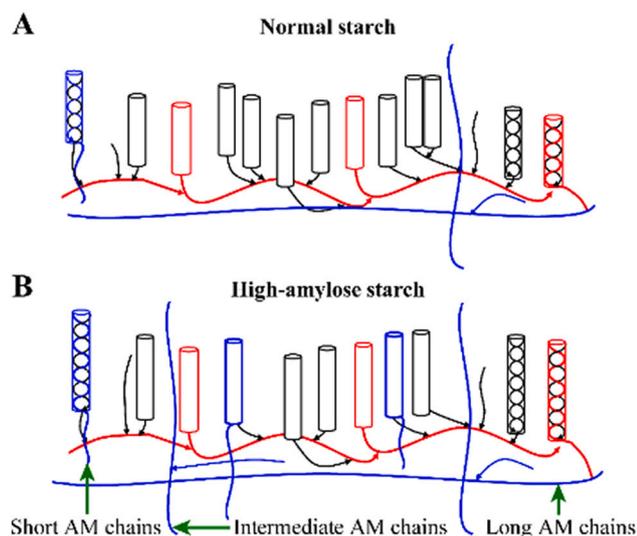


Fig. 2. Different AM fractions with different chain lengths contribute differently to the lamellar structure of normal starch and HAS granules. Cylinders denote double-helical segments, black lines are AP chain segments, red lines are AP backbone segments, blue lines are AM chain segments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lamellae and the AM content were found for legume and maize systems, respectively. When the AM was further increased to 99%, no lamellar peak was detected (Goldstein et al., 2016), showing that AM cannot form a regular nanostructure in the granule without the presence of AP. Plausible locations of AM in the chain grid of the cluster model has been suggested (Koroteeva, Kiseleva, Krivandin, et al., 2007; Koroteeva, Kiseleva, Sriroth, et al., 2007; Yuryev et al., 2004). However, AM has not been modelled within the building block backbone model and effects on the AP chain structure is still lacking. Plausible effects of AM based on the latter model is given in Fig. 2. However, it must be emphasized that the function of different AM fractions fits the cluster model as well.

3. Biosynthesis of HAS

3.1. Overview of normal starch biosynthesis

Starch granules are accumulated in chloroplasts as transient carbohydrate in autotrophic photosynthetic tissue, and as long-term storage carbohydrate in amyloplasts in heterotrophic tissue. Starch biosynthesis is a highly regulated process coordinately catalyzed by multiple enzymes (Pfister & Zeeman, 2016; Skryhan, Gurrieri, Sparla, Trost, & Blennow, 2018). The first step in starch biosynthesis is the conversion of glucose-1 phosphate (Glc1P) to the activated glucosyl donor ADP-glucose using ATP and catalyzed by the ADP-glucose pyrophosphorylase (AGPase). Subsequently, granule-bound starch synthases (GBSSs) and, to some extent, starch branching enzymes (SBEs) are responsible

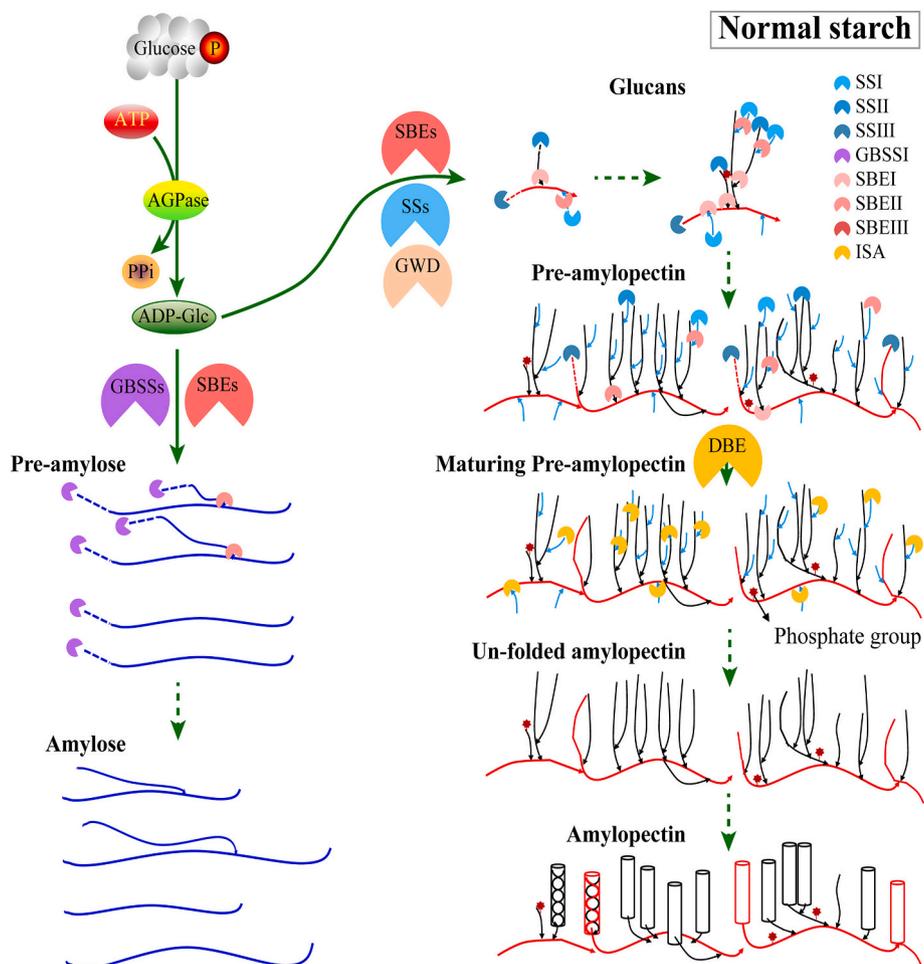


Fig. 3. The AM and AP biosynthesis pathways of normal starch. AM is synthesized primarily by the elongation of α -1,4-linked glucan chains and α -1,6-glucosidic transfer is scarce. AP is synthesized from glucans generating pre-AP with disordered branching. Subsequently these are trimmed into mature but non-helical AP, which is finally assembled to form packed double helical lamellae. Chain color codes are similar to those in Fig. 2.

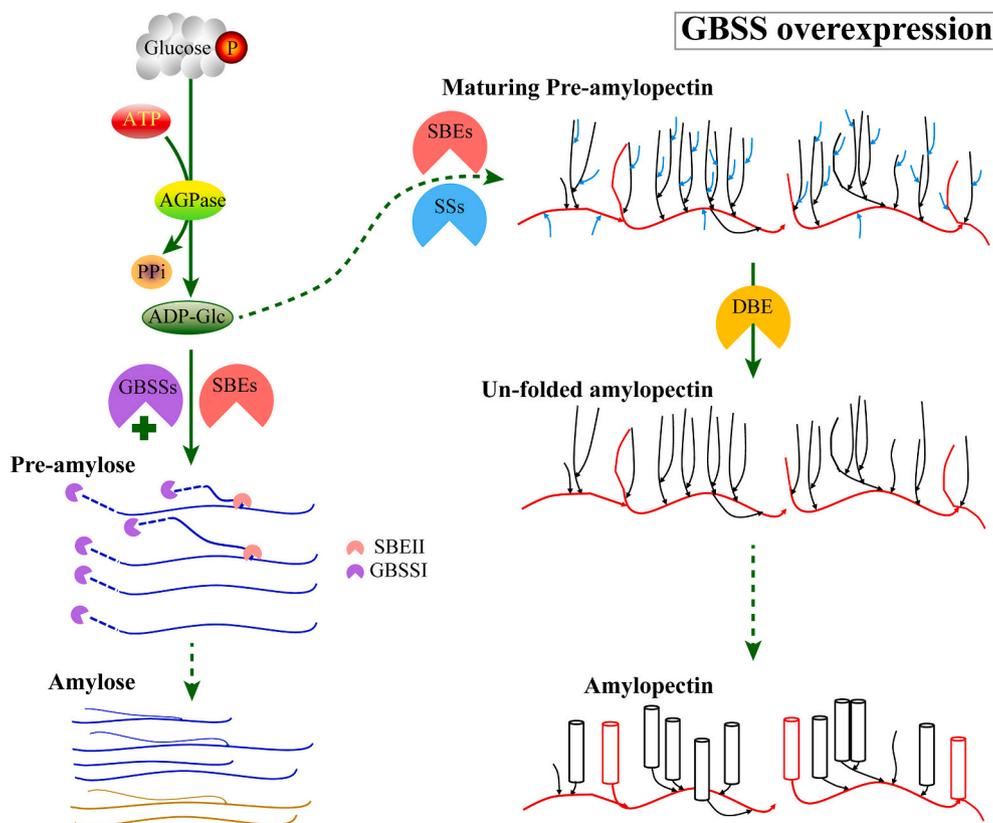


Fig. 4. The AM and AP biosynthesis pathways of GBSS overexpressor lines. Higher GBSS expression results in the generation of more AM molecules by boosting the AM biosynthesis metabolic branch. Chain color codes as in Fig. 2. AM molecules labelled brown denote superfluous AM generated due to the overexpression of GBSS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for AM biosynthesis, while soluble starch synthases (SSs), SBEs, starch debranching enzymes (DBEs), and glucan water dikinase (GWDs) plays different roles in AP biosynthesis.

To some extent GBSS, the main enzyme of AM biosynthesis, also interacts catalytically with SBEs introducing few branches in the AM molecules (Fig. 3). SSs are mainly involved in the synthesis of AP, and SSI, SSII, and SSIII are generally associated with the elongation of short, medium, and long AP chains, respectively (Fujita et al., 2007; Tetlow & Bertoft, 2020; Zhang et al., 2008). However, the annotations and substrate specificities of SSs for different chain lengths vary across species. For example, SSI has optimal catalytic activity for AP chains with DP 6–15 and DP 6–7 in maize (PL, 1998) and rice (Fujita et al., 2006), respectively. The SSI and SSII isoforms can be regarded as constituting the building blocks and short external segments according to the building block backbone representation, which form the clusters according to the cluster model (Tetlow & Bertoft, 2020). Generally SBEI has a preference for catalyzing the transfer of relatively long-chain segments (DP < 30, with the majority being DP 10–13), and the SBEII homologue has a preference for branched chains as a substrate and transfers shorter chains (DP 6–14) (Tetlow & Bertoft, 2020; Tetlow & Emes, 2014). The isoamylase-type debranching enzymes (ISAs) (ISA1/ISA2) are mainly responsible for pre-AP “trimming” or “scavenging” processes where, according to the cluster model, misplaced α -1,6 branch points are selectively removed to promote the formation of parallel double-helices and crystallization in the starch granule. This removes closely positioned chains on the backbone to provide flexibility for double helical segments to form (Bertoft, 2017; Hussain et al., 2003; Sim et al., 2014). DBE deficiency is suggested to result in continued action of the SSs and BEs on a population of polysaccharides that fail to crystallize, thereby causing formation of both pre-AP (also known as phytyloglycogen) and AP as end-products of a diverging pathway (Myers,

Morell, James, & Ball, 2000). In rice and kidney bean, the pullulanase-type debranching enzyme (PUL) has partially overlapping functions with ISA (Fujita et al., 2009; Takashima et al., 2007). Glucan water dikinases (GWDs) are associated with the phosphorylation of storage starch in tubers and roots, like potato and sweet potato, by a dikinase-type reaction mechanism in which the β -phosphate of ATP is transferred to either the C-6 or the C-3 position of the glucosyl residue of starch (Mikkelsen, Mutenda, Mant, Schürmann, & Blennow, 2005).

Recent investigations suggest that starch biosynthesis is a highly regulated process coordinately catalyzed by multiple enzymes involving phosphorylation-dependent multi-enzyme complexes (Crofts, Nakamura, & Fujita, 2017). The direct evidence for this is the presence of a SSI-SBEIIa-SBEIIb complex in wheat (Tetlow et al., 2008) and a SSI-SBEIIa-SBEIIb complex in maize (Liu et al., 2012). However, these complexes are rather dynamic in their activities and constitutions (e.g. the Tetlow & Bertoft, 2020 paper). The different starch structures found for mutants in identical genes are likely related to its specific composition of the starch biosynthesis protein complexes. For example, SSI-SSIIa-SBEIIb complexes and SSI-SSIIa-SBEI-SBEIIa complexes have been identified in the biosynthesis of normal starch maize and SBEIIb-deficient maize mutants (Liu et al., 2009). Moreover, loss of the SSIIIa homologue in rice is suggested to disrupt the SSIIIa-AGPase-SSIIa-SBEIIa-SBEIIb complexes during starch biosynthesis, thereby decreasing the AGPase activities affecting the starch structure and AM content (Zhou et al., 2016). Hence, complete knock-out of a polypeptide in such complexes can have severe disruptive effects on the biosynthetic machinery. Also, a non-functional enzyme within the complexes might have a very different effect compared to complete removal of that polypeptide. So far, most complexes consist of SSs, SBEs, and starch phosphorylase (Hennen-Bierwagen et al., 2008; Liu et al., 2011), and there is no evidence indicating that DBEs are part of protein complexes.

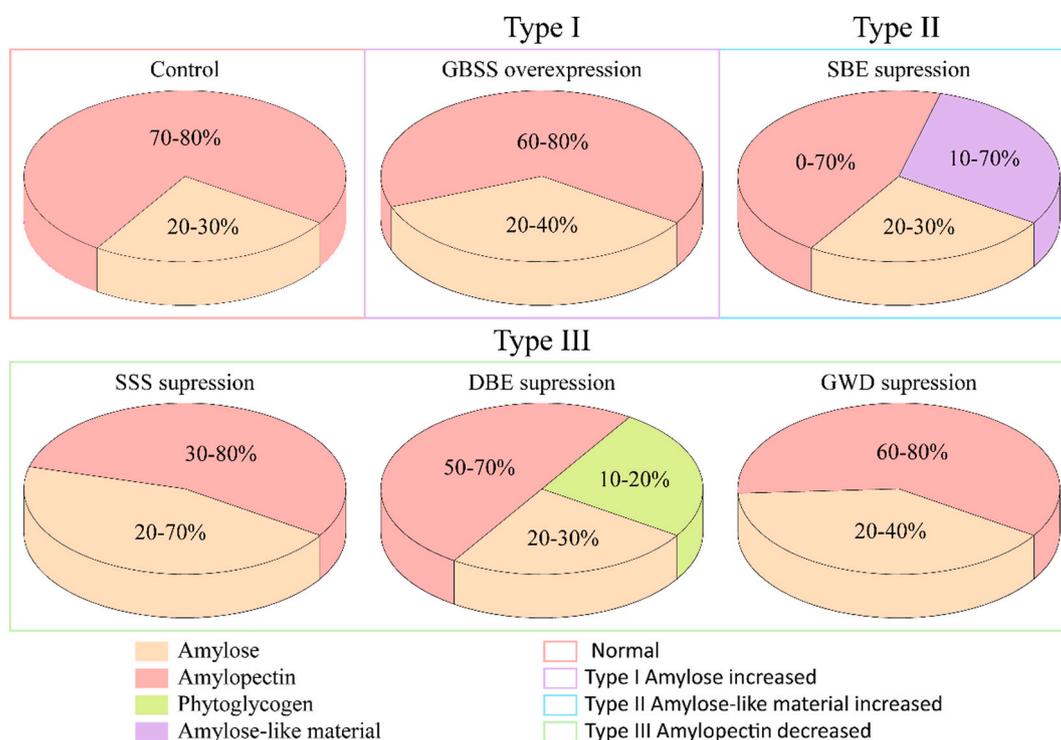


Fig. 5. The classification of different types of HAS by five types of different AM generation strategies and the corresponding AM range in different crops. The variations of AM content and ‘AM-like’ materials in these mutants indicate the different effects of various breeding techniques, e.g., TILLING and CRISPR, on enhancing AM content of starch from different botanical sources.

On the other hand, biosynthesis mathematical models based on AP chain-length distribution profiles (Wu & Gilbert, 2010) strongly suggest that the final chain length distributions of AP molecules are driven by the ratios of activities of Ss, SBEs, and DBEs.

In DBE-deficient mutants, the accumulation of phytoglycogen occurs, and the contents of starch and AP significantly decreased (Ball et al., 1996; Shannon, Garwood, & Boyer, 2009). This suggests that DBE is required for pre-amylopectin to attain a crystallization-competent structure (Myers et al., 2000). DBEs may trim highly branched polysaccharides (so-called pre-AP) generated by Ss-SBEs complexes until the lowest order structure suitable for crystallization is achieved. Hence, from those data there is circumstantial evidence that, in AP biosynthesis, Ss and SBEs generate pre-AP which is highly branched but disordered, and DBEs tailor the unnecessary branches in such pre-AP and generate a more ordered structure for following crystallization (Fig. 3).

3.2. The strategies to increase AM content by engineering starch biosynthesis pathways

According to the current knowledge of the starch biosynthesis pathway, three strategies can increase the AM content in starch: i) Increased content of ‘native’ AM by overexpression of GBSSs (Fig. 4) (Itoh et al., 2003; Sestili et al., 2012); ii) Production of ‘AM-like’ material in AP biosynthesis by suppressing SBEs (Fig. 6) (Carciofi et al., 2012; Zhong, Liu, Qu, Li, et al., 2020); and iii) Increased relative AM content by decreasing the AP content through suppressing Ss (Fig. 6), DBEs (Fig. 7), and GWDs (Fig. 7) (Blennow et al., 2020; Hogg et al., 2013; Kozlov et al., 2007; Li, Manghwar, et al., 2019).

Provided that GBSS is a limiting activity in the starch granule, overexpression of GBSSs is expected to increase the amounts of AM (Fig. 4). However, AM content is not always positively correlated with the GBSS activity (Flipse, Keetels, Jacobsen, & Visser, 1996). In some examples AM content was unchanged or only slightly increased (AM content <40%) in GBSS overexpressors of potato, wheat, and rice (Fig. 5) (Flipse et al., 1996; Itoh et al., 2003; Sestili et al., 2012).

Suppressing SBEs is the most common strategy to generate HASs in different species, including barley (Carciofi et al., 2012), wheat (Li, Dhital, Gilbert, & Gidley, 2020), maize (Jiang et al., 2015) and other starch crops (Fig. 6). Using this strategy, AM content increased to nearly 100% in the barley (Carciofi et al., 2012) and the potato (Zhao et al., 2021a, 2021b) systems, and typically increased to 70%–80% in maize (Jiang et al., 2015; Zhong, Liu, Qu, Blennow, et al., 2020) and wheat (Li, Dhital, Gilbert, & Gidley, 2020) (Fig. 5). Suppressing SBEs, especially the SBEIIb homologue, produces an α -glucan with only few branches attached to partial backbone chains, providing efficient substrates for the SSIII homologue for successive elongation. Possibly GBSS is also active on those linear segments. Such activities can produce material that we term ‘AM-like’ material in AP biosynthesis (Fig. 6) (Zhong, Liu, Qu, Li, et al., 2020). Such ‘AM-like’ material from AP biosynthesis and normal AM from AM biosynthesis, therefore together contributes to the overall AM content in these plants. In comparison with normal AM, ‘AM-like’ α -glucans are suggested to have more branches due to low expression of SBEs in plant unless SBEs are completely knocked out. We suggest that there are two possible ways to verify the existence of such ‘AM-like’ material: i), Engineering crops by simultaneously suppressing SBEs and GBSSs produce starch with ‘AM-like’ material and without normal AM. Isolated pure ‘AM-like’ material from these mutants can then be structurally compared with those of normal AM; and ii) Due to the proposed relatively high content of ‘AM-like’ material in extremely high AM starches (e.g., AM-only barley starch), all AM from such starches could be isolated and compared with the AM from normal starch. We adopted the second approach by testing the iodine binding capacity of AMs isolated from AM-only and normal barley starch, and potato starch, and found that AM isolated from AM-only barley starch showed lower iodine binding capacity than AM isolated from normal barley and potato starch (Fig. S1), which supports the existence of such ‘AM-like’ material. There may also be another component in HASs: so-called intermediate material, with a similar molecular size to that of AM but with a highly branched structure like AP. It may be that ‘AM-like’ material with few branches results in its greater capacity to form

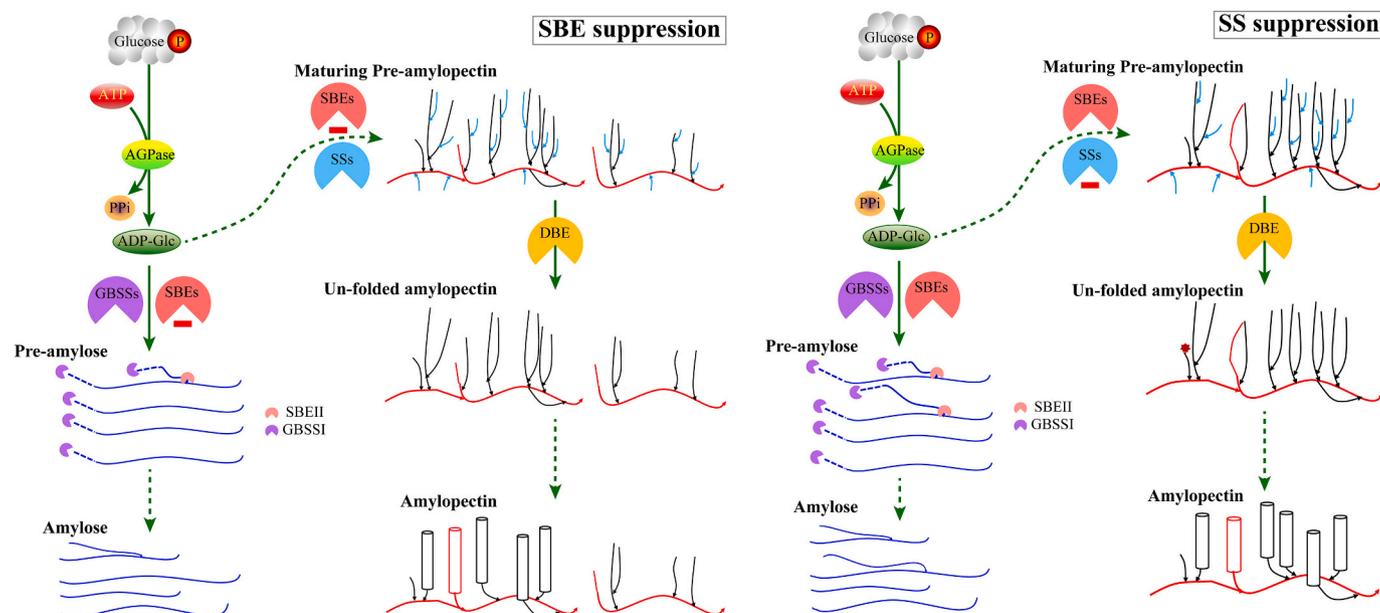


Fig. 6. The AM and AP biosynthesis pathways of SBE and SS suppressed mutants. Lower SBE expression results in the reduction of branch amounts in AP thereby generating ‘AM-like’ material in AP biosynthesis pathway. Lower SS expression results in the decrease of the amounts of AP molecules thereby increasing the relative AM content. Chain color codes as in Fig. 2.

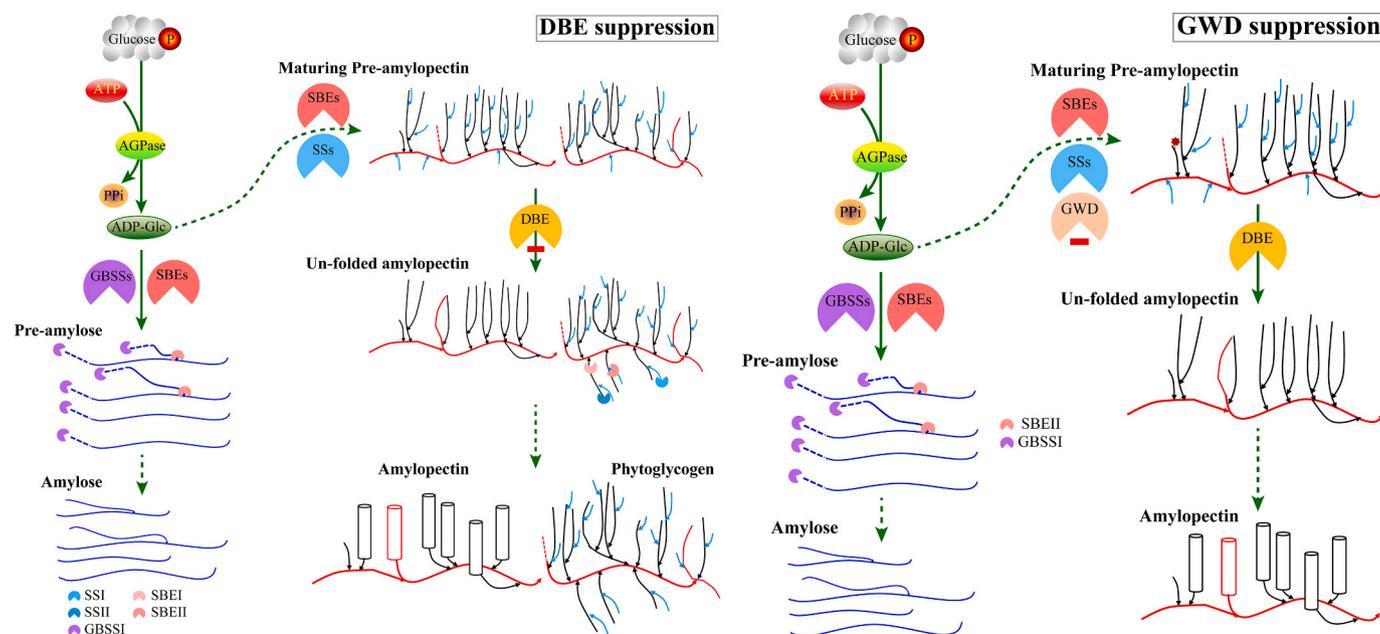


Fig. 7. The AM and AP biosynthesis pathways of DBE and GWD suppressed mutants. Lower DBE expression prevented the transformation from pre-AP with disordered branching points to AP with ordered branching points thereby generating more phytoglycogen and reducing the amounts of AP molecules in AP biosynthesis pathway. Lower GWD expression results in a decrease of the amounts of AP molecule thereby increasing the relative AM content. However, the mechanism behind this is still unclear. Chain color codes are similar to those in Fig. 2.

complexes with iodine which is similar to AM generated from GBSSs. Highly branched structures of intermediate material may then contribute to its higher hydrodynamic radius compared with that of ‘AM-like’ material as detected by gel permeation chromatography or size exclusion chromatography.

Suppressing SSSs induces the reduction of premature, highly branched AP, (i.e., pre-AP) to AP, thereby decreasing the relative content of AP and increasing the relative content of AM (Fig. 6). Through this strategy, the AM content was increased to 71% in barley starch (Li, Manghwar, et al., 2019; Morell et al., 2003), 38% in maize (Zhang et al., 2004), and

44% in wheat (Konik-Rose et al., 2007). Suppressing DBEs promotes the deposition of phytoglycogen (an intermediate material termed pre-AP) in AP biosynthesis (Fig. 7) (Blennow et al., 2020; Boyer & Preiss, 1981; Wong et al., 2003). This decreases the relative content of AP in starch. To our knowledge, the AM content can reach 38% by suppressing DBEs in potato (Blennow et al., 2020) and 30% in cassava (Ceballos et al., 2008). Therefore we estimate that the increased phytoglycogen content is equal to the increase of AM content (Fig. 5), although the accumulation of phytoglycogen in the DBE-deficit mutant can be much higher (Wong et al., 2003). Although GWD1 is mainly responsible for

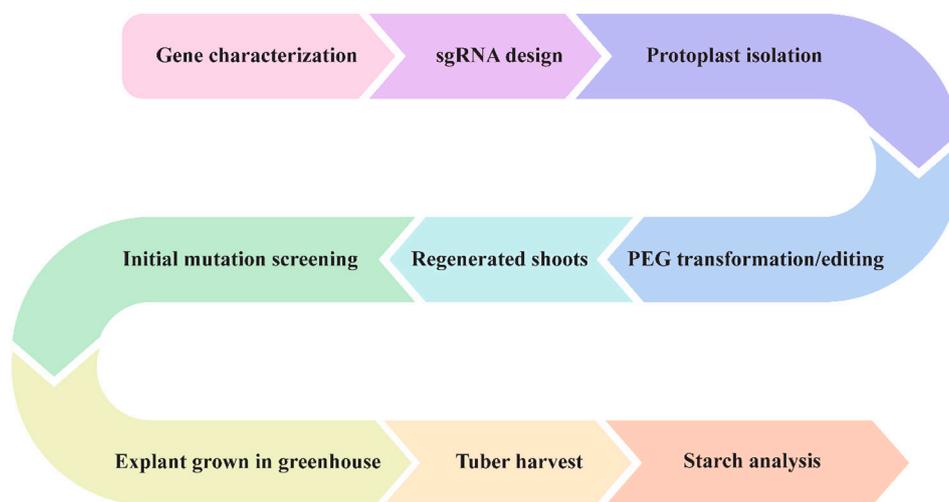


Fig. 8. Scheme for HAS potato generated by CRISPR/Cas (Bennett et al., 2020; Zhao et al., 2021a, 2021b).

the attachment of phosphate monoesters on C6 position of starch molecules (Mikkelsen et al., 2005), the phosphorylation of starch is suggested to promote the AP biosynthesis (Xu et al., 2017). Hence, similar to SSs, suppression of GWD can also suppress the AP biosynthesis, thereby decreasing AP content (Fig. 7). However, the suppression of GWD only causes a slight increase in AM content from 31% to 37% in potato (Fig. 5) (Kozlov et al., 2007). The underlying mechanism of how GWD specifically affects AP biosynthesis is still unknown.

4. Techniques for HAS engineering

4.1. Two main techniques for the generation of HAS cultivars

HAS cultivars may be generated by tweaking AM and AP biosynthesis by two techniques: conventional breeding and new breeding technologies (NBTs).

Many HAS crops, including the commercially available maize and rice HAS, have been generated by conventional breeding, often using random mutagenesis to produce a high-AM mutant using, e.g., ethyl methanesulfonate (EMS), sodium azide (NaN_3) or high-energy X-ray radiation, followed by phenotypic selection of the desired HAS trait and extensive crossing and backcrossing to further increase the AM content and improve the agronomic traits. Efficient high-throughput molecular screenings for identifying such mutations have been developed, including so-called Targeted Induced Local Lesions in Genomes (TILLING), a non-GM technique (Chen, Hao, Parry, Phillips, & Hu, 2014). For instance, high AM wheat lines were generated by such a TILLING system (Slade et al., 2012).

4.2. Precision breeding as a tool for engineering starch composition including HAS

Based on direct regulation of key biosynthetic enzymes in the crop storage organs, efficient molecular strategies are currently evolving (Blennow et al., 2013; Hebelstrup, Sagnelli, & Blennow, 2015), which include gene overexpression, gene silencing or specific point mutations using genome editing approaches like zinc-finger domain nucleases, TALEN factors or CRISPR/Cas (Hebelstrup et al., 2015).

Precise CRISPR/Cas-based breeding techniques are excellent strategies for HAS production to enable efficient introduction or elimination of desired or unwanted traits. The use of CRISPR/Cas-based breeding strategies is currently limited to a number of plant species and crops, which, however is growing (Hebelstrup et al., 2015). A number of requirements or challenges in terms of biological barriers need to be overcome, and further development of the technology as well as

regulatory demands and public accept must be addressed for precise crop breeding at a broader scale. Many starch crop plants have high ploidy and complex genomes, e.g., the tetraploid (starch) potato, with a high frequency of Single Nucleotide Polymorphisms (SNPs) that complicates application of CRISPR/Cas and the assessment/screening for edited cells and regenerated edited plants, since editing of all four alleles in a tetraploid species is normally required for obtaining a full loss of function phenotype (Hebelstrup et al., 2015). All of these challenges have been solved in the case of generating a potato line synthesizing pure AP (Johansen et al., 2019), and different HAS lines in starch crops, including rice (Guo et al., 2020; Sun et al., 2017), wheat (Li et al., 2021), sweet potato (Wang et al., 2019), and potato (Zhao et al., 2021a, 2021b). Generally, in HAS lines, CRISPR/Cas editing is employed to generate a full allelic loss-of-function of the targeted SBE genes in the plants (Guo et al., 2020; Li et al., 2021; Wang et al., 2019; Zhao et al., 2021a). Among these lines, the highest apparent AM content (100%) is reported for potato by editing both SBEI and SBEII (Zhao et al., 2021b).

A schematic protocol for generating HAS in potato from single cells using the CRISPR/Cas approach is shown in Fig. 8 (Bennett et al., 2020; Zhao et al., 2021a, 2021b). This requires the following steps: i) Single cell (protoplast) isolation and polyethylene glycol (PEG) mediated transformation of the CRISPR/Cas components, ii) Establishing plant regeneration protocols (which often varies between species and cultivars) and iii) reliable means of screening for editing protoplasts and regenerated plants (e.g. by the use of the InDel Detection Amplicon Analysis (IDAA) to detect small insertions or deletions) (Bennett et al., 2020; Johansen et al., 2019; Petersen et al., 2019).

Most often, breeding requires editing of several genes, which, if the traditional single-gene targeting strategy is applied, requires one round of plant regeneration for each gene that is edited (Hebelstrup et al., 2015). However, somaclonal mutations resulting from hormone usage in the regeneration from a single edited protoplast cell to edited plant is considerably higher than mutations derived from potential CRISPR off-target events and is sometimes visible at the phenotypic level ('distorted looking' plants or plant organs) (Li, Gidley, & Dhital, 2019). Therefore, previous single-gene targeting strategies must be replaced with multi-gene targeting strategies, so-called multiplexing where all or most of the desired genes are targeted in the same transformation event. However, this gives an increased screening load in the single plant regeneration step in which all copies of the desired genes must be edited.

To meet consumer demands for non-transgenic products, the use of DNA-free CRISPR/Cas, in the form of (Ribo)NucleoProteins (RNPs), alleviates risks of introducing transgenic DNA into the chromosomes.

Many challenges in relation to the introduction of precise breeding technologies on a broader scale for HAS breeding with high ploidy and

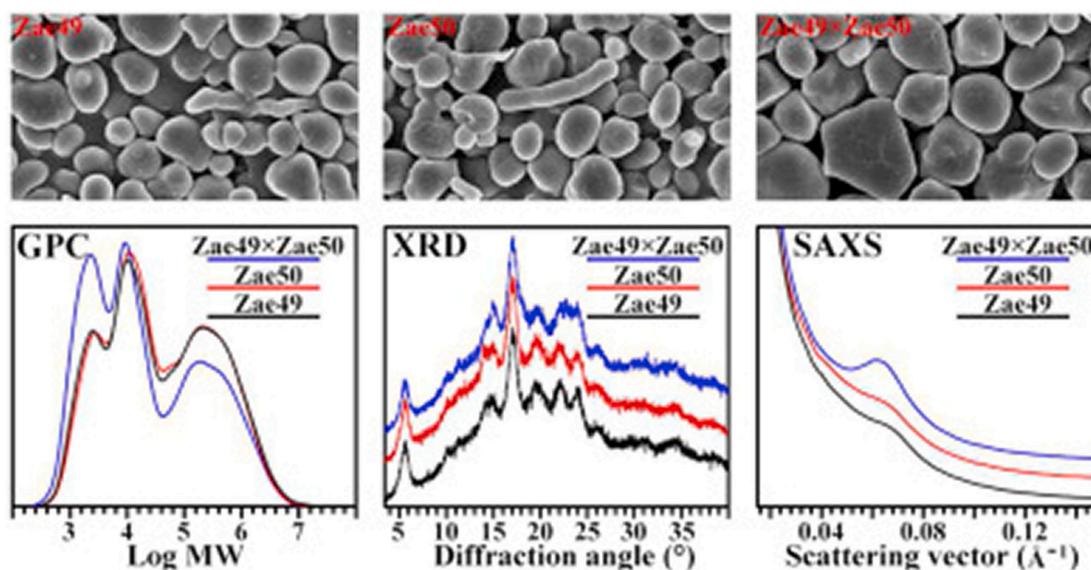


Fig. 9. The structural differences, including morphology by SEM (top panel), molecular structure (GPC), crystalline structure (XRD), and lamellar structure (SAXS) of inbred lines (Zae49 δ and Zae50 φ) and their F1 hybrid (Zae49 \times Zae50) of high AM maize (Lin, Guo, Zhao, et al., 2016).

complex genomes (Gao, 2018), have been met with efficient solutions, such as high throughput editing and screening techniques (Bennett et al., 2020; Johansen et al., 2019; Petersen et al., 2019). Because sexual propagation or backcrossing of some HAS crops (e.g., potato) leads to loss of existing qualities/traits, clonal amplification and propagation of edited off-spring is required. Protocols for regeneration of edited HAS plants are therefore important (Bennett et al., 2020; Johansen et al., 2019; Petersen et al., 2019).

4.3. Effect of genetic backgrounds on HAS breeding

A number of HAS varieties from different botanical sources, e.g., wheat, barley, maize, potato, pea, and rice, have been developed and characterized (Li, Manghwar, et al., 2019). Genetic backgrounds, such as inbred and hybrid background genotypes, are important for HAS and the structure and properties of the starch (Capek, Drábik, & Turjan, 2010; Liang, Wang, & Lu, 1996; Tongquan, 1998; Yuan, Zhu, Wang, & Zhang, 1994). HASs from hybrid genotypes with the same HAS mutant locus display very different structure and properties compared with HASs from their inbred line parents (Fig. 9) (Lin, Guo, Huang, et al., 2016; Lin, Guo, Zhao, et al., 2016). A recent study showed that two HAS genotypes mutated in the same locus, and having similar AM contents, displayed notable differences in molecular structure, lamellar structure, and digestibility (Zhong, Liu, Qu, Blennow, et al., 2020). Hence, different HAS varieties affected by the same genes can be very different with respect to their starch molecular structure and properties. Such effects remain to be characterized at the different structural levels of the starch granule.

In addition to genotypic characteristics, the environmental condition during growth is also an important factor affecting the biosynthesis of starch, thereby influencing the structure and function of starch. For example, the starch content in the normal wheat grain is affected by soil nitrogen and meteorological conditions during growth (Nhan & Copeland, 2014). Moreover, the growing season and location has been shown to affect the AM content, the proportion of short chains of AP and swelling power of wheat starch (Nhan & Copeland, 2014). It has been reported that water-deficiency significantly enhanced the abscisic acid (ABA) content in the grains, and that this was positively correlated with the activities of Ss and SBEs (Yang, Zhang, Wang, Xu, & Zhu, 2004). This showed that drought has an effect on starch molecular structure. However, little information is documented about the environmental

effects on the AM content, structure, or functionality of HAS-producing crops. Such information is essential for the large operations and industrial applications of HAS crops.

5. Future perspectives

This review covers biosynthesis, genetic engineering, breeding, and structure of HAS. Prospects are identified as follows: i) More data must be made available on the genetic backgrounds and environmental factors in relation to the structures and function of HAS; ii) How AM contributes to the crystalline and lamellar structure should be further clarified based on advanced techniques for analyzing AM fine structure and its crystalline and lamellar structure; iii) SBE-deficient HAS mutants produce AM-like material having functionality close to AM generated from the common AM biosynthesis pathway. However, this material must be isolated to permit a full structural and functional comparison to AM generated from the common AM biosynthesis pathway. Moreover, the biosynthetic route for AM-like material must be delineated; and iv) A comprehensive and systematic data repository of HAS from different cultivars should be established.

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Declaration of competing interest

The authors declare no competing financial interest.

References

- Amalala, A., Bateman, S., Dean, K., Petinakis, E., Sangwan, P., Wong, S., Leong, K., ... (2011). An overview of degradable and biodegradable polyolefins. *Progress in Polymer Science*, 36(8), 1015–1049.
- Ball, S., Guan, H.-P., James, M., Myers, A., Keeling, P., Mouille, G., Preiss, J., ... (1996). From glycogen to amylopectin: A model for the biogenesis of the plant starch granule. *Cell*, 86(3), 349–352.
- Bennett, E. P., Petersen, B. L., Johansen, I. E., Niu, Y., Yang, Z., Chamberlain, C. A., Frödin, M., ... (2020). INDEL detection, the ‘Achilles heel’ of precise genome editing: A survey of methods for accurate profiling of gene editing induced indels. *Nucleic Acids Research*, 48(21), 11958–11981.
- Bertoft, E. (2017). Understanding starch structure: Recent progress. *Agronomy*, 7(3), 56.
- Bertoft, E., Koch, K., & Åman, P. (2012). Building block organisation of clusters in amylopectin from different structural types. *International Journal of Biological Macromolecules*, 50(5), 1212–1223.
- Blazek, J., & Gilbert, E. P. (2011). Application of small-angle X-ray and neutron scattering techniques to the characterisation of starch structure: A review. *Carbohydrate Polymers*, 85(2), 281–293.
- Blennow, A., Jensen, S. L., Shaik, S. S., Skryhan, K., Carciofi, M., Holm, P. B., Tanackovic, V., ... (2013). Future cereal starch bioengineering: Cereal ancestors encounter gene technology and designer enzymes. *Cereal Chemistry*, 90(4), 274–287.
- Blennow, A., Skryhan, K., Tanackovic, V., Krunic, S. L., Shaik, S. S., Andersen, M. S., Nielsen, K. L., ... (2020). Non-GMO potato lines, synthesizing increased amylose and resistant starch, are mainly deficient in isoamylase debranching enzyme. *Plant Biotechnology Journal*, 18(10), 2096–2108.
- Boyer, C. D., & Preiss, J. (1981). Evidence for independent genetic control of the multiple forms of maize endosperm branching enzymes and starch synthases. *Mechanisms of Saccharide Polymerization & Depolymerization*, 67(6), 1141–1145.
- Capek, P., Drábik, M., & Turjan, J. (2010). Characterization of starch and its mono and hybrid derivatives by thermal analysis and FT-IR spectroscopy. *Journal of Thermal Analysis and Calorimetry*, 99(2), 667–673.
- Carciofi, M., Blennow, A., Jensen, S. L., Shaik, S. S., Henriksen, A., Buléon, A., & Hebelstrup, K. H. J. B. P. B. (2012). *Concerted suppression of all starch branching enzyme genes in barley produces amylose-only starch granules*. 12(1). BMC Plant Biology, 223–223.
- Ceballos, H., Sánchez, T., Denyer, K., Tofiño, A. P., Rosero, E. A., Dufour, D., Fahy, B., ... (2008). Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). *Journal of Agricultural and Food Chemistry*, 56(16), 7215–7222.
- Chen, L., Hao, L., Parry, M. A., Phillips, A. L., & Hu, Y. G. (2014). Progress in TILLING as a tool for functional genomics and improvement of crops. *Journal of Integrative Plant Biology*, 56(5), 425–443.
- Chen, X., & Yan, N. (2020). A brief overview of renewable plastics. *Materials Today Sustainability*, 7–8, Article 100031.
- Crofts, N., Nakamura, Y., & Fujita, N. (2017). Critical and speculative review of the roles of multi-protein complexes in starch biosynthesis in cereals. *Plant Science*, 262, 1–8.
- Dureja, H., Khatak, S., Khatak, M., & Kalra, M. (2011). Amylose rich starch as an aqueous based pharmaceutical coating material-Review. *International Journal of Pharmaceutical Sciences and Drug Research*, 3(1), 08–12.
- Flipse, E., Keetels, C., Jacobsen, E., & Visser, R. (1996). The dosage effect of the wildtype GBSS allele is linear for GBSS activity but not for amylose content: Absence of amylose has a distinct influence on the physico-chemical properties of starch. *Theoretical and Applied Genetics*, 92(1), 121–127.
- Follain, N., Joly, C., Dole, P., & Bliard, C. (2005). Mechanical properties of starch-based materials. I. Short review and complementary experimental analysis. *Journal of Applied Polymer Science*, 97(5), 1783–1794.
- French, D. (1972). Fine structure of starch and its relationship to the organization of starch granules. *Journal of the Japanese Society of Starch Science*, 19(1), 8–25.
- Fujita, N., Toyosawa, Y., Utsumi, Y., Higuchi, T., Hanashiro, I., Ikegami, A., Inomata, K., ... (2009). Characterization of pullulanase (PUL)-deficient mutants of rice (*Oryza sativa* L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. *Journal of Experimental Botany*, 60(3), 1009–1023.
- Fujita, N., Yoshida, M., Asakura, N., Ohdan, T., Miyao, A., Hirochika, H., & Nakamura, Y. (2006). Function and characterization of starch synthase I using mutants in rice. *Plant Physiology*, 140(3), 1070–1084.
- Fujita, N., Yoshida, M., Kondo, T., Saito, K., Utsumi, Y., Tokunaga, T., Jane, J. L., ... (2007). Characterization of SSIIa-deficient mutants of rice: The function of SSIIa and pleiotropic effects by SSIIa deficiency in the rice endosperm. *Plant Physiology*, 144(4), 2009–2023.
- Gao, C. (2018). The future of CRISPR technologies in agriculture. *Nature Reviews Molecular Cell Biology*, 19(5), 275–276.
- Goldstein, A., Annor, G., Putaux, J.-L., Hebelstrup, K. H., Blennow, A., & Bertoft, E. (2016). Impact of full range of amylose contents on the architecture of starch granules*. *International Journal of Biological Macromolecules*, 89, 305–318.
- Guo, D., Ling, X., Zhou, X., Li, X., Wang, J., Qiu, S., Zhang, B., ... (2020). Evaluation of the quality of a high-resistant starch and low-glutelin Rice (*Oryza sativa* L.) generated through CRISPR/Cas9-mediated targeted mutagenesis. *Journal of Agricultural and Food Chemistry*, 68(36), 9733–9742.
- Hebelstrup, K. H., Sagnelli, D., & Blennow, A. (2015). The future of starch bioengineering: GM microorganisms or GM plants? *Frontiers in Plant Science*, 6, 247.
- Hennen-Bierwagen, T. A., Liu, F., Marsh, R. S., Kim, S., Gan, Q., Tetlow, I. J., Myers, A. M., ... (2008). Starch biosynthetic enzymes from developing maize endosperm associate in multisubunit complexes. *Plant Physiology*, 146(4), 1892–1908.
- Hogg, A., Gause, K., Hofer, P., Martin, J., Graybosch, R. A., Hansen, L., & Giroux, M. (2013). Creation of a high-amylose durum wheat through mutagenesis of starch synthase II (SSIIa). *Journal of Cereal Science*, 57(3), 377–383.
- Hussain, H., Mant, A., Seale, R., Zeeman, S., Hinchliffe, E., Edwards, A., Martin, C., ... (2003). Three isoforms of isoamylase contribute different catalytic properties for the debranching of potato glucans. *The Plant Cell*, 15(1), 133–149.
- Itoh, K., Ozaki, H., Okada, K., Hori, H., Takeda, Y., & Mitsui, T. (2003). Introduction of wx transgene into rice wx mutants leads to both high- and low-amylose rice. *Plant and Cell Physiology*, 44(5), 473–480.
- Jiang, H., Campbell, M., Wu, Y., Du, S., Srichuwong, S., & Jane, J. L. (2015). Dosage effect of high-amylose modifier gene(s) on the starch structure of maize amylose-extender mutant. *Journal of Agricultural & Food Chemistry*, 63(2), 433.
- Johansen, I. E., Liu, Y., Jørgensen, B., Bennett, E. P., Andreasson, E., Nielsen, K. L., Petersen, B. L., ... (2019). High efficacy full allelic CRISPR/Cas9 gene editing in tetraploid potato. *Scientific Reports*, 9(1), 1–7.
- Kainuma, K., & French, D. (1971). Naegeli amyloextrin and its relationship to starch granule structure. I. Preparation and properties of amyloextrins from various starch types. *Biopolymers: Original Research on Biomolecules*, 10(9), 1673–1680.
- Kainuma, K., & French, D. (1972). Naegeli amyloextrin and its relationship to starch granule structure. II. Role of water in crystallization of B-starch. *Biopolymers: Original Research on Biomolecules*, 11(11), 2241–2250.
- Konik-Rose, C., Thistleton, J., Chanvrier, H., Tan, I., Halley, P., Gidley, M. L., Z., ... (2007). Effects of starch synthase IIa gene dosage on grain, protein and starch in endosperm of wheat. *Theoretical and Applied Genetics*, 115(8), 1053.
- Koroteeva, D. A., Kiseleva, V. I., Krivandin, A. V., Shatalova, O. V., Biasczak, W., Bertoft, E., Yuryev, V. P., ... (2007). Structural and thermodynamic properties of rice starches with different genetic background: Part 2. Defectiveness of different supramolecular structures in starch granules. *International Journal of Biological Macromolecules*, 41(5), 534–547.
- Koroteeva, D. A., Kiseleva, V. I., Sriroth, K., Piyachomkwan, K., Bertoft, E., Yuryev, V. P., & Yuryev, V. P. (2007). Structural and thermodynamic properties of rice starches with different genetic background: Part 1. Differentiation of amylopectin and amylose defects. *International Journal of Biological Macromolecules*, 41(4), 391–403.
- Kozhevnikov, G. O., Protserov, V. A., Wasserman, L. A., Pavlovskaya, N. E., Golischkin, L. V., Milyaev, V. N., & Yuryev, V. P. (2001). Changes of thermodynamic and structural properties of wrinkled pea starches (Z-301 and paramagnet varieties) during biosynthesis. *Starch-Stärke*, 53(5), 201–210.
- Kozlov, S. S., Krivandin, A. V., Shatalova, Olga, V., & Bertoft, E. (2006). Structure of starches extracted from near-isogenic wheat lines: Part 2. Molecular organization of amylopectin clusters (regular). *Journal of Thermal Analysis & Calorimetry*, 86(2), 291–301.
- Kozlov, S. S., Blennow, A., Krivandin, A. V., & Yuryev, V. P. (2007). Structural and thermodynamic properties of starches extracted from GBSS and GWD suppressed potato lines. *International Journal of Biological Macromolecules*, 40(5), 449–460.
- Laohaphatanaleart, K., Piyachomkwan, K., Sriroth, K., & Bertoft, E. (2010). The fine structure of cassava starch amylopectin: Part 1: Organization of clusters. *International Journal of Biological Macromolecules*, 47(3), 317–324.
- Li, C., Dhital, S., Gilbert, R. G., & Gidley, M. J. (2020). High-amylose wheat starch: Structural basis for water absorption and pasting properties. *Carbohydrate Polymers*, 245, Article 116557.
- Li, H., Dhital, S., Flanagan, B. M., Mata, J., Gilbert, E. P., & Gidley, M. J. (2020). High-amylose wheat and maize starches have distinctly different granule organization and annealing behaviour: A key role for chain mobility. *Food Hydrocolloids*, 105820.
- Li, H., Gidley, M. J., & Dhital, S. (2019). High-amylose starches to bridge the “Fiber Gap”: Development, structure, and nutritional functionality. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 362–379.
- Li, J., Jiao, G., Sun, Y., Chen, J., Zhong, Y., Yan, L., Xia, L., ... (2021). Modification of starch composition, structure and properties through editing of TaSBEIIa in both winter and spring wheat varieties by CRISPR/Cas9. *Plant Biotechnology Journal*, 19(5), 937–951.
- Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Rui, H., ... (2019). Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. *Plant Biotechnology Journal*, 17(5), 858–868.
- Liang, J., Wang, X., & Lu, N. (1996). Scanning electron microscopic observation on starch grains of hybrid rice and their parents. *Zhongguo Shuidao Kexue*, 10(2), 79–84.
- Lin, L., Guo, D., Huang, J., Zhang, X., Zhang, L., & Wei, C. (2016). Molecular structure and enzymatic hydrolysis properties of starches from high-amylose maize inbred lines and their hybrids. *Food Hydrocolloids*, 58, 246–254.
- Lin, L., Guo, D., Zhao, L., Zhang, X., Wang, J., Zhang, F., & Wei, C. (2016). Comparative structure of starches from high-amylose maize inbred lines and their hybrids. *Food Hydrocolloids*, 52, 19–28.
- Liu, F., Ahmed, Z., Lee, E. A., Donner, E., Liu, Q., Ahmed, R., Tetlow, I. J., ... (2011). Allelic variants of the amylose extender mutation of maize demonstrate phenotypic variation in starch structure resulting from modified protein-protein interactions. *Journal of Experimental Botany*, 63(3), 1167–1183.
- Liu, F., Makhmoudova, A., Lee, E. A., Wait, R., Ems, M. J., & Tetlow, I. J. (2009). The amylose extender mutant of maize conditions novel protein-protein interactions between starch biosynthetic enzymes in amyloplasts. *Journal of Experimental Botany*, 60(15), 4423–4440.
- Liu, F., Romanova, N., Lee, E. A., Ahmed, R., Evans, M., Gilbert, E. P., Tetlow, I. J., ... (2012). Glucan affinity of starch synthase IIa determines binding of starch synthase I and starch-branching enzyme IIb to starch granules. *Biochemical Journal*, 448(3), 373–387.
- Matveev, Y. I., Van Soest, J., Nieman, C., Wasserman, L., Protserov, V., Ezernitskaja, M., & Yuryev, V. (2001). The relationship between thermodynamic and structural

- properties of low and high amylose maize starches. *Carbohydrate Polymers*, 44(2), 151–160.
- Mikkelsen, R., Mutenda, K. E., Mant, A., Schürmann, P., & Blennow, A. (2005). α -glucan, water dikinase (GWD): A plastidic enzyme with redox-regulated and coordinated catalytic activity and binding affinity. *Proceedings of the National Academy of Sciences*, 102(5), 1785–1790.
- Moorthy, S. N., Andersson, L., Eliasson, A. C., Santacruz, S., & Ruales, J. (2006). Determination of amylose content in different starches using modulated differential scanning calorimetry. *Starch-Stärke*, 58(5), 209–214.
- Morell, M. K., Kosar-Hashemi, B., Cmiel, M., Samuel, M. S., Chandler, P., Rahman, S. Li, Z., ... (2003). Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *The Plant Journal*, 34(2), 173–185.
- Myers, A. M., Morell, M. K., James, M. G., & Ball, S. G. (2000). Recent progress toward understanding biosynthesis of the amylopectin Crystal1. *Plant Physiology*, 122(4), 989–998.
- Nhan, M. T., & Copeland, L. (2014). Effects of growing environment on properties of starch from five Australian wheat varieties. *Cereal Chemistry*, 91(6), 587–594.
- Nikuni, Z. (1978). Studies on starch granules. *Starch-Stärke*, 30(4), 105–111.
- Perera, A., Meda, V., & Tyler, R. (2010). Resistant starch: A review of analytical protocols for determining resistant starch and of factors affecting the resistant starch content of foods. *Food Research International*, 43(8), 1959–1974.
- Petersen, B. L., Möller, S. R., Mravec, J., Jørgensen, B., Christensen, M., Liu, Y., Yang, Z., ... (2019). Improved CRISPR/Cas9 gene editing by fluorescence activated cell sorting of green fluorescence protein tagged protoplasts. *BMC Biotechnology*, 19(1), 1–12.
- Pfister, B., & Zeeman, S. C. (2016). Formation of starch in plant cells. *Cellular and Molecular Life Sciences*, 73(14), 2781–2807.
- PL, K. (1998). Starch biosynthesis: Understanding the functions and interactions of multiple isozymes of starch synthase and branching enzyme. *Trends in Glycoscience and Glycotechnology*, 10(54), 307–319.
- Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). In, 5(1). *Resistant starch—A review* (pp. 1–17).
- Sestili, F., Botticella, E., Proietti, G., Janni, M., D'Ovidio, R., & Lafiandra, D. (2012). Amylose content is not affected by overexpression of the wx-B1 gene in durum wheat. *Plant Breeding*, 131(6), 700–706.
- Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances*, 26(3), 246–265.
- Shannon, J. C., Garwood, D. L., & Boyer, C. D. (2009). Genetics and physiology of starch development. *Starch*, 23–82.
- Sim, L., Beeren, S. R., Findinier, J., Dauvillée, D., Ball, S. G., Henriksen, A., & Palčić, M. M. (2014). Crystal structure of the Chlamydomonas starch debranching enzyme isoamylase ISA1 reveals insights into the mechanism of branch trimming and complex assembly. *Journal of Biological Chemistry*, 289(33), 22991–23003.
- Skryhan, K., Gurrieri, L., Sparla, F., Trost, P., & Blennow, A. (2018). Redox regulation of starch metabolism. *Frontiers in Plant Science*, 9, 1344.
- Slade, A. J., McGuire, C., Loeffler, D., Mullenberg, J., Skinner, W., Fazio, G., Goodstal, J. F., ... (2012). Development of high amylose wheat through TILLING. *BMC Plant Biology*, 12(1), 69.
- Sterling, C. (1974). Fibrillar structure of starch. Evidence for crossed fibrils from incipient gelatinization. *Starch-Stärke*, 26(4), 105–110.
- Sun, Y., Jiao, G., Liu, Z., Zhang, X., Li, J., Guo, X., Zhao, Y., ... (2017). Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Frontiers in Plant Science*, 8, 298.
- Takashima, Y., Senoura, T., Yoshizaki, T., Hamada, S., Ito, H., & Matsui, H. (2007). Differential chain-length specificities of two isoamylase-type starch-debranching enzymes from developing seeds of kidney bean. *Bioscience Biotechnology & Biochemistry*, 71(9), 2308–2312.
- Tetlow, I. J., Beisel, K. G., Cameron, S., Makhmoudova, A., Liu, F., Bresolin, N. S., Emes, M. J., ... (2008). Analysis of protein complexes in wheat amyloplasts reveals functional interactions among starch biosynthetic enzymes. *Plant Physiology*, 146(4), 1878–1891.
- Tetlow, I. J., & Bertoff, E. (2020). A review of starch biosynthesis in relation to the building block-backbone model. *International Journal of Molecular Sciences*, 21(19), 7011.
- Tetlow, I. J., & Emes, M. J. (2014). A review of starch-branching enzymes and their role in amylopectin biosynthesis. *IUBMB Life*, 66(8), 546–558.
- Tongquan, S. Y. L. Y. (1998). Effect of different glutinous genotypes of maize endosperm to the content of total and branched chain starch [J]. *Journal of Beijing Agricultural College*, 4.
- Wang, H., Wu, Y., Zhang, Y., Yang, J., Fan, W., Zhang, H., Zhang, P., ... (2019). CRISPR/Cas9-based mutagenesis of starch biosynthetic genes in sweet potato (*Ipomoea Batatas*) for the improvement of starch quality. *International Journal of Molecular Sciences*, 20(19), 4702.
- Waigh, T. A., Perry, P., Riekel, C., Gidley, M. J., & Donald, A. M. (1998). Chiral side-chain liquid-crystalline polymeric properties of starch. *Macromolecules*, 31(22), 7980–7984.
- Wong, K.-S., Kubo, A., Jane, J.-L., Harada, K., Satoh, H., & Nakamura, Y. (2003). Structures and properties of amylopectin and phytylglycogen in the endosperm of sugary-1 mutants of Rice. *Journal of Cereal Science*, 37(2), 139–149.
- Wu, A. C., & Gilbert, R. G. (2010). Molecular weight distributions of starch branches reveal genetic constraints on biosynthesis. *Biomacromolecules*, 11(12), 3539–3547.
- Xu, X., Dees, D., Dechesne, A., Huang, X.-F., Visser, R. G., & Trindade, L. M. (2017). Starch phosphorylation plays an important role in starch biosynthesis. *Carbohydrate Polymers*, 157, 1628–1637.
- Yamaguchi, M., Kainuma, K., & French, D. (1979). Electron microscopic observations of waxy maize starch. *Journal of Ultrastructure Research*, 69(2), 249–261.
- Yang, J., Zhang, J., Wang, Z., Xu, G., & Zhu, Q. (2004). Activities of key enzymes in sucrose-to-starch conversion in wheat grains subjected to water deficit during grain filling. *Plant Physiology*, 135(3), 1621–1629.
- Yuan, L., Zhu, Q., Wang, Z., & Zhang, Z. (1994). Preliminary observation on the properties of starch granules in endosperm of hybrid rice between subspecies and their parents. *Journal of Jiangsu Agricultural College*, 15(2), 45–50.
- Yuryev, V. P., Krivandin, A. V., Kiseleva, V. I., Wasserman, L. A., Genkina, N. K., Fornal, J., Schiraldi, A., ... (2004). Structural parameters of amylopectin clusters and semi-crystalline growth rings in wheat starches with different amylose content. *Carbohydrate Research*, 339(16), 2683–2691.
- Zhang, X., Colleoni, C., Ratushna, V., Sirghie-Colleoni, M., James, M. G., & Myers, A. M. (2004). Molecular characterization demonstrates that the zea mays gene sugary2 codes for the starch synthase isoform SSIIa. *Plant Molecular Biology*, 54(6), 865–879.
- Zhang, X., Szydlowski, N., Delvallé, D., D'Hulst, C., James, M. G., & Myers, A. M. (2008). Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in Arabidopsis. *BMC Plant Biology*, 8(1), 96, 8, 1(2008–09–23).
- Zhao, X., Jayarathna, S., Turesson, H., Fält, A.-S., Nestor, G., González, M. N., Andersson, R., ... (2021). Amylose starch with no detectable branching developed through DNA-free CRISPR-Cas9 mediated mutagenesis of two starch branching enzymes in potato. *Scientific Reports*, 11(1), 1–13.
- Zhao, X., Jayarathna, S., Turesson, H., Fält, A.-S., Nestor, G., González, M. N., Andersson, R., ... (2021). Amylose starch with no detectable branching developed through DNA-free CRISPR-Cas9 mediated mutagenesis of two starch branching enzymes in potato. *Scientific Reports*, 11(1), 4311.
- Zhong, Y., Liang, W., Pu, H., Blennow, A., Liu, X., & Guo, D. (2019). Short-time microwave treatment affects the multi-scale structure and digestive properties of high-amylose maize starch. *International Journal of Biological Macromolecules*, 137, 870–877.
- Zhong, Y., Liu, L., Qu, J., Blennow, A., Hansen, A. R., Wu, Y., Liu, X., ... (2020). Amylose content and specific fine structures affect lamellar structure and digestibility of maize starches. *Food Hydrocolloids*, 105994.
- Zhong, Y., Liu, L., Qu, J., Li, S., Blennow, A., Seytahmetovna, S. A., Guo, D., ... (2020). The relationship between the expression pattern of starch biosynthesis enzymes and molecular structure of high amylose maize starch. *Carbohydrate Polymers*, 116681.
- Zhou, H., Wang, L., Liu, G., Meng, X., Jing, Y., Shu, X., Smith, S. M., ... (2016). Critical roles of soluble starch synthase SSIIIa and granule-bound starch synthase waxy in synthesizing resistant starch in rice. *Proceedings of the National Academy of Sciences*, 113(45), 12844–12849.