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Comparative proteome analysis of A- and B-type starch granule-associated proteins in bread wheat (*Triticum aestivum* L.) and *Aegilops crassa*

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ABSTRACT

Starch is the main component in the wheat endosperm and exists in two forms including A- and B-type granules. A bread wheat line *CB037A* and an *Aegilops* line *Aegilops crassa* were studied for the underlying starch biosynthesis mechanism in relation to granule types. The wheat line contains both types of starch granules while the *Aegilops* line only has the A-type. Differential starch granule development patterns of these two species were observed at the morphological level. A total of 190 differentially expressed proteins (DEPs) were detected between the two lines based on 2-D electrophoresis, among which 119 DEPs were identified, representing 13 unique proteins. Gene ontology annotation analysis indicated that both molecular functions and biological processes of the identified proteins are highly conserved. Different phosphorylation modification levels between the A- and B-type starch granules were found. Real-time quantitative reverse transcription PCR analysis revealed that a number of key genes including *starch synthase I-1*, *pullulanase*, *isoamylase* and *starch branching enzyme IIa* were differentially expressed between the two species. Our results demonstrated that the large granule size is associated with higher activities of multiple starch biosynthesis enzymes. The phosphorylation of starch biosynthesis enzymes is related with the formation of B-type starch granules.

Abbreviations: SSI-1, starch synthase I-1; SSIIa-1, starch synthase IIa-1; SSIIa-3, starch synthase IIa-3; GBSS, granule bond starch synthase; GBSSI, granule bond starch synthase I; SBEI, starch branching enzyme I; SBEIIa, starch branching enzyme IIa; SBEIIb, starch branching enzyme IIb; AGPase small subunit, adenosine 5′ diphosphate glucose pyrophosphorylase small subunit; AGPase larger subunit, adenosine 5′ diphosphate glucose pyrophosphorylase larger subunit; Pull, pullulanase; Iso, isoamylase; ADPGlc, adenosine 5′ diphosphate glucose; DP, degree of polymerization; DPA, days post anthesis; SEM, scanning electron microscope; RDS, rapidly digestible starch; SDS, slowly digestible starch; MALDI-TOF/TOF-MS, matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry; DEPs, differentially expressed proteins; GO, gene ontology; CBB, Coomassie brilliant blue; qRT-PCR, real-time quantitative reverse-transcription polymerase chain reaction.

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Biological significance

Analyzed the proteome, transcriptome and phosphorylation of core starch granule biosynthesis enzymes and provided new insights into the differential mechanisms underlying the A- and B-type starch granule biosyntheses.

1. Introduction

Starch is the main component in the wheat endosperm that accounts for about 70% of dry seed weight. It is the main source of energy for human consumption which represents up to 80% of daily caloric intake. Starch variation also has a wide range of impacts on human health [1–3]. World annual starch production from cereals is approximately 2050 million tonnes [4], which are widely used in fields such as plastic, pharmacy, building, and textile [5–8].

Starch molecule deposits in the starch granule as a semi-crystalline structure [9,10]. Based on their diameters, starch granules can be classified into three types, A-type (diameters greater than 10 μm), B-type (diameters between 5 and 10 μm), and C-type (diameters less than 5 μm). However, the B-type and C-type are usually combined together as B-type starch granules (diameters less than 10 μm) [11–13]. The A-type starch granules show a lenticular shape that is formed starting from 4 days post anthesis during grain development and continues to increase in size until the end of grain-filling period. While, the B-type starch granules show a spherical shape that is initiated at 11 days post anthesis and maintains the small size until the end of grain-filling [14,15].

Starch granules are mainly composed of amylose and amylopectin. Amylose is a kind of polysaccharides with a linear structure of alpha-(1–4) linked D-glucose units; whereas amylopectin is a type of polysaccharides that has highly branched structure of short alpha-(1–4) chains linked by alpha-(1–6) bonds. The starch granule biosynthesis is a complex process that involves a serial of enzymes, mainly including adenosine 5′ diphosphate glucose pyrophosphorylase (AGPase), starch synthase (SS), granule bound starch synthase (GBSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) [16]. The biosynthesis procedure of starch granule starts from the AGPase functioning which is responsible for the production of adenosine 5′ diphosphate glucose (ADPGlc) that is the soluble precursor and substrate for starch synthases [17–19]. Then starch synthase (SS) begins to extend the starch chain by synthesizing alpha-(1–4) bond. The starch synthase includes two big groups, SS and GBSS. SS contains four isoforms, SSI, SSII, SSIII, and SSIV [20]. Among these, SSI is primarily responsible for the shortest glucan chain synthesis with its DP (degree of polymerization) less than 10 glucosyl units [21]. SSII can be further divided to SSIIa and SSIIb [22,23]. In monocots, SSIIa is specific to extension of short chains (DP ≤ 10 glucosyl units), which is synthesized by SSI, to form intermediate-size chains with DP ranging from 12 to 24 glucosyl units [24]. The GBSS is encoded by the *Waxy* locus in cereals and is responsible for elongation of amylose [25,26]. GBSS can also be further separated to GBSSI that primarily appears in storage tissues and GBSSII that mostly presents in the leaves and other non-storage tissues and is responsible for the synthesis of transient starch [27–32]. Apart from main glucan chain, starch also contains branch chain that is synthesized by SBE and DBE. SBE is the enzyme that synthesizes the branch chains of the amylopectin molecule by two steps. Firstly, SBEs cleave internal alpha-(1–4) bonds to release a short oligosaccharide chain; secondly, the short oligosaccharide chain is transferred to branch chain linked by alpha-(1–6) bond and forms the branch chain [33–35]. After these two steps, some improper branches are removed by starch debranching enzymes such as isozymes and pullulan that efficiently hydrolyze alpha-(1–6)-linkages in amylopectin [36–39]. The biosynthesis of starch granules is the result of the coordinated actions of all enzymes described above that begins with the immediate soluble precursor of ADPGlc and ends with the formation of a starch granule [16].

Protein post-translational modification is a widespread phenomenon in plants, which is the most common type among various modifications. Recent studies revealed that phosphorylation also occurs in starch granule biosynthesis, suggesting that starch metabolic enzymes located in granules are regulated by post-translational modification and/or protein–protein interactions [40,41].

Previous evidence supports the hypothesis that starch biosynthesis in the cereal endosperm is coordinated by unique combinations of multiple isoforms of AGPase, starch synthase (including SS and GBSS) as well as branching and debranching enzymes. However, details about the complex cooperation among the above described synthases remain unclear [42–44]. Two main challenges in starch research are in two aspects. Firstly, it is difficult to extract and separate pure A- and B-type starch granules from cereal endosperm. Secondly, it is difficult to remove the saccharides and harvest enough proteins from the starch granules for proteomics study due to the high content of sugar and very low content of protein in the granules [11].

Recently, the relationship between starch properties and human health has attracted a wide attention. The nutritional quality of starch strongly depends on its structure [45]. The digestion of starch in the human small intestine is a complex process [46], of which the main factor affecting starch digestibility is attributed to the ratio of amylose:amylopectin [2]. The ratio of amylase:amylopectin in the A-type starch granules is higher than that in the B-type starch granules, approximately 30% and 25%, respectively [47]. In addition, due to the structure difference, the A-type starch granules have a higher susceptibility to hydrolysis than that of the B-type starch granules [48–50]. The A-type starch granule is more easily digestible than the B-type starch granule since it contains more short double helices than the B-type starch granule [48,51,52]. Depending on the digestible property of starch granules, the starch can be classified to rapidly digestible starch (RDS) and slowly digestible starch (SDS) [45]. The RDS is rapidly digested, resulting in an unstable postprandial blood glucose level, often sharp increase and fast decline in comparison with SDS that is usually associated with a sustained level of postprandial blood glucose due to slow digestion [53]. In