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Roles of GBSSI and SSIIa in determining amylose fine structure

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ABSTRACT

This study examines the relationships between genetics (single nucleotide polymorphisms (SNPs) in *GBSSI* and *SSIIa* genes), starch structure (amylose and amylopectin fine structures), and starch properties (relating to gelatinization). *GBSSI* and *SSIIa* SNPs did not alter the starch content of rice grains. *GBSSI* SNPs can affect the amylose content, but they are incapable of altering the chain-lengths of amylopectin and amylose. The amounts of both long and short amylose branches changed with the same trend as amylose content, and they appeared to affect starch gelatinization properties. SSIIa synthesizes intermediate single-lamella amylopectin chains (DP 16–21), and consequently impacts the gelatinization temperature. Mathematical modelling suggests that the reduction in SSIIa activity significantly increases the activity of SBEII, resulting in a decreased activity ratio of SS to SBE in the enzyme set governing an appropriate chain-length distribution range. This application of the genetics-structure-property paradigm provides selection strategies to produce rice varieties with improved qualities.

1. Introduction

Amylopectin and amylose are branched glucose polymers which are the two main components of starch. The glucose units are linked via α-(1→4) glycosidic linkages and branched via α-(1→6) glycosidic linkages. Amylose has only a few long-chain branches, while amylopectin is of much higher molecular weight, and hyperbranched with a large number of short chains. Past studies have found the significance of the fine structure of amylopectin and amylose, including amylose content and chain length distributions (CLDs), on the functional properties of starch, such as pasting, gelatinization and digestion rate (Tester & Morrison, 1990).

Starch is synthesized in higher plants through a complex pathway regulated by multiple starch-synthesizing enzymes, the major ones of which are starch synthases, starch branching enzymes (SBE) and starch debranching enzymes (DBE). Starch synthases catalyze the addition of ADP-glucose onto the non-reducing ends of a starch molecule and are divided into two types: granule-bound starch synthases (GBSS) and soluble starch synthases (SS) (Wang, Henry, & Gilbert, 2014). Multiple isoforms exist for each type of enzyme. For instance, four classes of SS have been identified: SSI, SSII (including SSIIa, SSIIb and SSIIc), SSIII and SSIV, while two GBSS isoforms, GBSSI and GBSSII, have been reported (Ball & Morell, 2003). *GBSSI* and *SSIIa* genes are believed to have major single-nucleotide polymorphisms (SNPs) that influence the properties of rice starch (Kharabian-Masouleh, Waters, Reinke, Ward, & Henry, 2012; Tian et al., 2009).

GBSSI is encoded by the *waxy* gene in cereals, and it is primarily responsible for the synthesis of linear chains in amylose (Smith, Denyer, & Martin, 1997). Several SNPs in *GBSSI* gene have been characterized and associated with variations in amylose content and starch properties in rice. A T/G SNP at the junction site of intron 1 and exon 1 is able to distinguish rice genotypes with low amylose content from those with intermediate and high amylose contents (Cai, Wang, Xing, Zhang, & Hong, 1998). Another functional C/A SNP

Abbreviations: ANOVA, analysis of variance; APTS, 8-aminopyrene-1,3,6,trisulfonate acid; AUC, area under the curve; CLD, chain length distribution; DBE, starch debranching enzymes; DMSO, dimethyl sulfoxide; DP, degree of polymerization; DSC, differential scanning calorimeter; FACE, fluorophore-assisted carbohydrate electrophoresis; GBSS, granule bound starch synthases; SBE, starch branching enzymes; SEC, size-exclusion chromatography; SNP, single nucleotide polymorphism; SS, starch synthases.

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in exon 6 that results in a tyrosine/serine substitution has the ability to discriminate intermediate and high amylose contents (Larkin & Park, 2003). Rice cultivars with intermediate amylose contents have a C at this location, while cultivars with high amylose contents have either A or C. Apart from the two major SNPs that could explain 86.7% of the differences in amylose content (Chen, Bergman, Pinson, & Fjellstrom, 2008a), another C/T SNP in exon 10 has also been identified in high amylose content rice cultivars, and has been related to the pasting properties (Chen, Bergman, Pinson, & Fjellstrom, 2008b; Larkin, McClung, Ayres, & Park, 2003) and gel consistency (Tran et al., 2011) of rice flour. So far, studies have been mainly focused on the influences of *GBSSI* alleles on the amylose content and pasting/gel properties of starch. However, the relationships between *GBSSI* alleles, amylose CLDs and the starch properties are not yet clear.

SSIIa, an SSII isoform, predominantly expressed in cereal endosperms, is believed to be involved in amylopectin synthesis (Yamamori, Fujita, Hayakawa, Matsuki, & Yasui, 2000). It has been proposed that the difference in SSIIa activity is responsible for the distinction between the amylopectin structure of indica-type rice and that of japonica-type rice, where indica-type rice has higher SSIIa activity than japonica-type rice, leading to a higher proportion of amylopectin chains of DP ~ 12–24 chains and a lower proportion of DP < 11 chains (Nakamura et al., 2005; Umemoto, Yano, Satoh, Shomura, & Nakamura, 2002). Such differences impact on rice cooking quality and starch gelatinization properties (Umemoto et al., 2008). Significant polymorphisms in the exon 8 of *SSIIa* gene have been identified, and they were reported to be critical for SSIIa function and starch gelatinization properties (Cuevas et al., 2010; Kharabian-Masouleh et al., 2012; Waters, Henry, Reinke, & Fitzgerald, 2006). However, it is not known if the *SSIIa* alleles have impacts on the CLDs of long amylopectin chains (DP > 70) and amylose chains, which have significant influences on starch gelatinization properties.

Although several past studies have reported the functions of *GBSSI* and *SSIIa* in influencing key starch physiochemical properties (such as pasting, gelatinization, retrogradation and texture properties) (Lu et al., 2010; Yang et al., 2014), amylose content (Chen et al., 2008b) and the CLD of short amylopectin chains (Umemoto et al., 2002), current knowledge on the roles of *GBSSI* and *SSIIa* in controlling the CLDs of amylose and long amylopectin chains (which could not be precisely characterized in the past) is still limited. In addition, the relationships between the CLDs of amylose and amylopectin and the gelatinization properties of starch are not yet fully understood. The logical sequence on which this present paper is based on the paradigm that the *genetics* determine *starch structure*, which in turn influences *properties* such as gelatinization temperature. This paradigm inserts the missing link between the results discussed above, which are between genetics and properties. The objective of this study is to improve understanding of the roles of starch synthesizing enzymes GBSSI and SSIIa in controlling the synthesis of amylose and long amylopectin chains, and to establish the first causal relationships among (a) key SNPs in *GBSSI* and *SSIIa* genes, (b) CLDs of amylose and long amylopectin chains, and (c) the gelatinization properties of rice starches.

The molecular structures of starch from a set of rice breeding lines with different SNPs in *GBSSI* and *SSIIa* genes were characterized by size-exclusion chromatography, SEC, and fluorophore-assisted carbohydrate electrophoresis, FACE. Associating SNPs with the fine structure of rice starches can provide information on the roles of GBSSI and SSIIa in determining amylose and amylopectin fine structure, improving understanding of the biosynthetic pathway of starch in rice. This analysis may reveal relationships that provide selection strategies to produce rice varieties with higher yields while also improved quality.

2. Materials and methods

2.1. Materials

Seven temperate (japonica-type) F6 rice breeding lines with various alleles (Table 1) in their *GBSSI* and *SSIIa* genes were obtained from Yanco Agricultural Institute, Department of Primary Industries, NSW, Australia. SNPs in the starch genes in these samples were reported by Kharabian-Masouleh et al. (2012). Protease from *Streptomyces griseus* (type XIV) was purchased from Sigma–Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). Isoamylase (from *Pseudomonas sp.*), amyloglucosidase (from *Aspergillus niger*) and D-Glucose (GOPOD Format) kit were purchased from Megazyme International Ltd. (Bray, Co. Wicklow, Ireland). A series of pullulan standards with peak molecular weights ranging from 342 to 2.35×10^6 were purchased from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was purchased from Merck Co. Inc. (Kilsyth, VIC, Australia). All other chemicals were reagent grade and used as received.

2.2. Starch content of rice grains

Rice grains were manually dehulled and ground into fine flour using a cryo-mill (Freezer/Mill 6850 SPEX, Metuchen, NJ, USA) in a liquid nitrogen bath. The milling was carried out for 5 min at 10 s^{-1}. The starch content of rice grains was analyzed from the cryo-milled rice flour using the Megazyme Total Starch (AA/AMG) assay kit following a method described by Li, Hasjim, Dhital, Godwin, and Gilbert (2011).

2.3. Starch extraction for structure characterization

The finely ground rice flour was treated following an established method (Syahariza, Li, & Hasjim, 2010) to extract starch. A protease and sodium bisulfite solution was used first, followed by a centrifugation step, to remove protein from the rice flour. The treated rice flour was agitated in DMSO containing 0.5% (w/w) LiBr

Table 1
Single nucleotide polymorphisms (SNPs) in *GBSSI* and *SSIIa* genes of rice breeding lines.

Sample No.	Sample ID	*GBSSI*			*SSIIa*		Pedigree
		EX/IN1[a]	EX6	EX10	Ref4827	Ref4828ER	
14	YRR08_03_02	T&T[b]	A&A	C&C	G&T	T&C	YRB4 (SANT ANDREA/M7//VIALONE/LADY WRIGHT)
38	YRI08_04_10	G&T	A&A	C&C	G&G	C&C	YRL113//L203/YRL34
108	YRA08_03_10	T&T	A&A	C&C	T&T	T&T	KOSHIHIKARI/M102//YRM43
109	YRA08_03_11	T&T	A&A	C&C	G&G	C&C	YRK4/SR13925-13-1
159	YRB08_04_10	G&G	C&C	C&C	G&G	C&C	M201//YR196/ARDITO///YRM54
192	YUD08_01_22	G&G	A&A	T&T	G&G	C&C	L205
223	YRD08_05_05	T&T	A&A	C&C	T&T	T&T	YRL101/4/YRL39///213D.25/YR83//M7/IRR.INGA

[a] EX stands for exon and IN stands for intron. EX/IN1 is the junction site of exon1 and intron 1.
[b] The nucleotides at the same location of a pair of chromosomes.