

REVIEW ARTICLE

Genetic controls on starch amylose content in wheat and rice grains

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Abstract

Starch accumulates in plants as granules in chloroplasts of source organs such as leaves (transitory starch) or in amyloplasts of sink organs such as seeds, tubers and roots (storage starch). Starch is composed of two types of glucose polymers: the essentially linear polymer amylose and highly branched amylopectin. The amylose content of wheat and rice seeds is an important quality trait, affecting the nutritional and sensory quality of two of the world's most important crops. In this review, we focus on the relationship between amylose biosynthesis and the structure, physical behaviour and functionality of wheat and rice grains. We briefly describe the structure and composition of starch and then in more detail describe what is known about the mechanism of amylose synthesis and how the amount of amylose in starch might be controlled. This more specifically includes analysis of GBSS alleles, the relationship between waxy allelic forms and amylose, and related quantitative trait loci. Finally, different methods for increasing or lowering amylose content are evaluated.

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Introduction

Wheat and rice are the world's two most economically important crops, contributing food for more than 90% of the world's population. Both crops have undergone extensive breeding to fuel the 'Green Revolution' (e.g. the introduction of semidwarf traits into rice and wheat from Chinese and Japanese varieties) (Makino 2011). Wheat is grown around the world and has the broadest adaptation of all the cereals (Posner 2000). The most widely cultivated wheat today is *Triticum aestivum* (bread wheat), subspecies *vulgare*, which is a hexaploid form created by the hybridization of diploid and tetraploid types (Posner 2000). This species has three homoeologous genomes (A, B and D), each including seven pairs of chromosomes (Feldman *et al.* 1995). A tetraploid wheat, called durum (macaroni) wheat (*Triticum durum*), is popular in pasta production (Dvorak *et al.* 1998) and has two genomes (A and B). The mature grain of wheat, after removing the husk, consists of the embryo and endosperm, both filial tissues, surrounded by a thin layer of maternal tissues comprising of the nucellus, testa and pericarp. The endosperm is made up of the outer aleurone layer and inner columns of starchy endosperm cells (Hoseney 2002). The pericarp plus testa, aleurone layer and embryo are

removed during milling, leaving starchy endosperm as the main contributor to wheat flour.

Rice accounts for 23% of the world's supply of calories (Brar and Khush 2002). The mature rice grain, after removal of the hull (husk), again consists of the embryo and the starchy endosperm, surrounded by the seed coat, comprised of remnant tissues from the nucellus, testa and pericarp (Drea *et al.* 2005). The seed coat, embryo and the aleurone layer from the bran is removed during the milling (often called polishing in rice) process. Most of the seed's protein and lipid are located in the rice bran (Fasahat *et al.* 2012a). The polished rice is essentially endosperm like wheat and consists of 90% starch (Smith *et al.* 1997). However, unlike wheat, rice is usually consumed as a whole grain.

Starch is a carbohydrate composed of two distinct types of glucose polymers, amylose and amylopectin. Amylose may be regarded as a long, essentially linear chain composed of $10^2 - 10^4$ D-glucosyl units joined by $\alpha(1 \rightarrow 4)$ linkages. The mean length is about 250–370 residues in rice (Juliano 1985; Vandeputte and Delcour 2004) and about the same in wheat (Takeda *et al.* 1984; Wang *et al.* 1998). The ratio of amylose to amylopectin in wheat endosperm is 1:3, similar to rice (Rahman *et al.* 2005).

Amylose is only moderately soluble and tends to form aggregates, leading to opaque solutions (Liu 2005). Amylose also contains infrequent $1 \rightarrow 6$ linked branches. In a

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study by Takeda *et al.* (1993) the structures of branched and linear molecules of the isolated amylose from defatted rice (*japonica*, Nipponbare) were examined. The branch number in rice amylose is 2–5 chains on average per molecule (Takeda *et al.* 1986). Wheat amylose also has an average branch number of about two per molecule (Wang *et al.* 1998).

Amylopectin in rice and wheat comprises about 70–80% of the total starch and is larger than amylose on average, being made of 10^4 – 10^5 glucose residues. These are linked $\alpha(1\rightarrow4)$, but branches occur every 20–25 residues because of the presence of $\alpha(1\rightarrow6)$ linkages (figure 1) (Vandeputte and Delcour 2004; Regina *et al.* 2006). Branch linkages are not positioned randomly and are organized in clustered structures in terms of linear A, B, and C chains (figure 2). The A chains, which do not carry any other chains, are linked to B chains which in turn are linked to a C chain containing the single reducing group (Manners 1989). Park *et al.* (2007) reported the average molecular mass of rice starch amylose were about 3.8×10^5 for long and medium grain nonwaxy rice. The values for amylopectin were 1.10×10^8 (long grain), 1.81×10^8 (short/medium grain), and 2.47×10^8 (waxy grain). These correspond to about 10^4 glucose residues for amylose and 10^6 for amylopectin. According to Zhong *et al.* (2006), the molecular mass of amylose and amylopectin were in the range $(3.1\text{--}3.4) \times 10^6$ and $(4.0\text{--}5.5) \times 10^7$, respectively, for the same rice categories. Clearly, there are some differences in the size estimates. The molecular mass for wheat have been estimated to be in a similar range (Yoo and Jane 2002; Ratnayake and Jackson 2007; Gumul *et al.* 2008). Not unexpectedly, the waxy starches appear to have larger amylopectin molecules and high amylose starches have a lower average degree of polymerization (Takeda *et al.* 1993; Yoo and Jane 2002).

Starch morphology and structure

Starch granule structure and morphology have been covered in a number of recent reports and the reader is referred to them for further details (Jenkins and Donald 1997; Denyer *et al.* 2001; Gérard *et al.* 2002; Pilling and Smith 2003; Vandeputte and Delcour 2004). The initiation of starch granules is still unclear. Mature rice starch granules are roughly cuboid in appearance and smaller than wheat or maize (figure 3; Kaur *et al.* 2007). The rice starch granules are

normally attached to each other in the grain and for this reason the rice starch granules are often said to be complex. Lack of phosphorylase in rice at low growth temperatures lead to smaller and less angular starch granules (Satoh *et al.* 2008). In contrast, the mature wheat endosperm has three class of granules (figure 3): the large lenticular A type granules (10–20 μm in diameter), and the much smaller spherical B type granules, and C type starch granules (less than 10 μm in diameter) (Meredith 1981; Eliasson and Karlsson 1983; Dengate and Meredith 1984; Parker 1985; Bechtel *et al.* 1990; Wei *et al.* 2010a). The C type granules are those with a mean diameter less than 5 μm and often irregular in shape (Bechtel *et al.* 1990; Wei *et al.* 2010a), while the A type starch granules represent the greatest proportion of endosperm starch by weight at maturity ($> 50\%$), the B and C types starch granule predominate numerically ($> 90\%$) (Evers and Lindley 1977; Stoddard 1999; Wei *et al.* 2010a); the proportions reported vary somewhat, depending on the investigators. Flours with starch comprising of only purified B granules have noticeably higher water absorption and longer mixing time compared with reconstituted flour containing only A granules (Rahman *et al.* 2000). The A type granules form about 4–7 days after anthesis and B type granules appear around 10–12 days after anthesis and the C types form later (Parker 1985; Bechtel *et al.* 1990).

All starch granules contain both crystalline and amorphous regions, and are therefore semicrystalline (figure 4). The semicrystalline granule consists of the aligned branches of amylopectin providing the crystalline region and the low branching regions of amylopectin and amylose comprising the amorphous region. The crystalline area can take different forms depending on the packing of the helical regions. These crystalline polymorphs are known as the A (the amylopectin chains are of 23–29 glucose residues), B (the amylopectin chains are of 30–44 residues), C (mixture of A and B forms) and V (resulting from complexing with lipids) (Wu and Sarko 1978a,b; Hosney 1994; Bhattacharya 2004). The starch of rice and wheat are usually of A form but can switch to B if the branching activity is suppressed (Butardo *et al.* 2011). The shape of the granule and characteristic of a plant species appears to be retained provided the amylose does not exceed a certain proportion of the starch (e.g., Regina *et al.* 2006; Wei *et al.* 2010b; Butardo *et al.* 2012); waxy starches appear normal (Jiranuntakul *et al.* 2011). However, very high amylose starches, in both wheat and rice, lead to abnormally

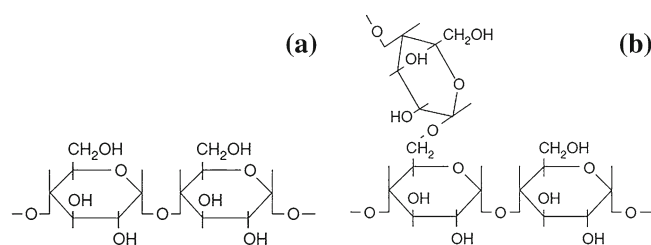


Figure 1. The structure of (a) amylose and (b) amylopectin (Liu 2005).

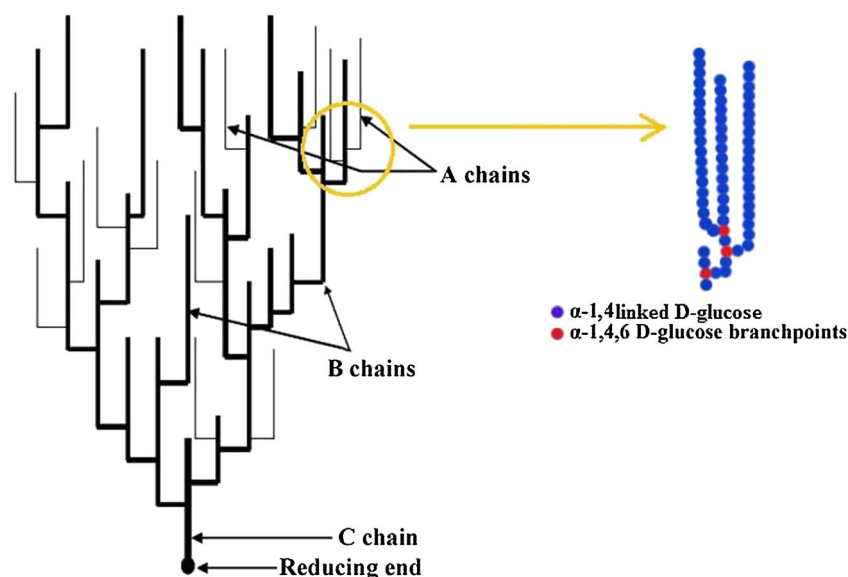


Figure 2. Amylopectin clustered structure based on Manners (1989).

shaped starch granules (Regina *et al.* 2006; Wei *et al.* 2010b,c).

There are reports that amylose content changes during development and it is concentrated in the inside of the starch granule. The evidence for this is provided by Lyon *et al.* (1999), Denyer *et al.* (2001), Zhang *et al.* (2003), Siebenmorgen *et al.* (2006) and Gealy and Bryant (2009).

Quality and the measurement of amylose

The proportion of amylose in rice and wheat has a large impact on its use and desirability, and the sensory properties of the grain are partly determined by the amylose content. Increased amylose in food is associated with increased resistant starch. Resistant starch is the starch that is fermented in the large colon because it is not broken down in the small intestine. There are different types of resistant starch depending on why the starch is resistant to digestion in the small intestine (Rahman *et al.* 2007). High amylose starch produce more resistant starch in the uncooked state and also after

cooking and cooling, but this effect is of significance at proportions of amylose greater than 50%. The fermentation of resistant starch in the large intestine has a number of health benefits (Higgins 2004). In a study by Regina *et al.* (2006), feeding rats with more than 70% amylose wheat wholemeal caused the improvement of several indices of large-bowel function compared with standard wholemeal wheat. The rate at which starch is broken down in the small intestine, leading to the release of glucose in the bloodstream, which is termed the GI, is also generally reduced by amylose content in rice (Fitzgerald *et al.* 2011; Karupiah *et al.* 2011), but this may not explain all the factors involved. Clearly, other components of the meal will also have an effect. Experiments with wheat indicate that for wheat products, the slow digestion in the small intestine is only noticeable when the amylose content rises above 38% (Hallstrom *et al.* 2011).

The accurate measurement of amylose is problematic. This is because 'amylose' and 'amylopectin' do not represent specific molecular structures but instead classes of molecules. Depending on the methods used to determine 'amylose' and 'amylopectin', different proportions of these

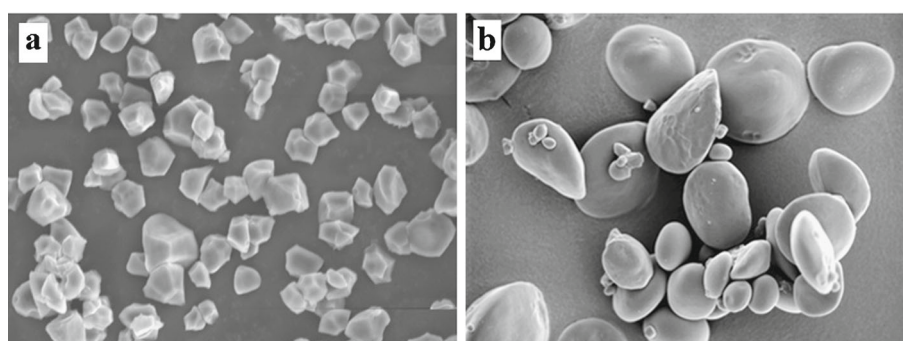


Figure 3. Scanning electron microscopy of rice (a, at 2000X) and wheat (b, at 850X) starch granules.

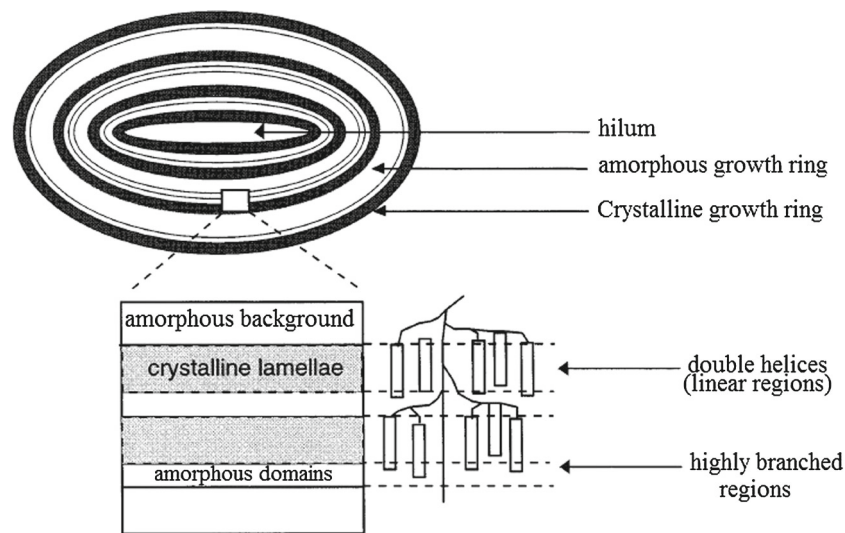


Figure 4. Starch granular structure: (a) the whole granule, (b) the lamellae, and (c) the polymer chains. Adapted from van der Burgt *et al.* (1999).

classes may fall into the designated categories. These methods have included colorimetry, gel-filtration, near-infrared spectroscopy and solubility (Mahmood *et al.* 2007; Zhu *et al.* 2008; Fitzgerald *et al.* 2009; Mukerjea and Robyt 2010). Further, resistant starch by definition is difficult to digest and can lead to inaccuracies in the measurement of total starch and amylose in assays performed *in vitro*.

Despite the issues alluded to above, commercial rice varieties are assorted into market classes, based on having waxy (0–2%), very low (2–10%), low (10–20%), intermediate (20–25%), and high (>25%) amylose (Juliano 1979). Amylose content is evaluated as percentage of starch and not as percentage of sample weight. Higher amylose cultivars (>25%) are prevalent in indica rice which correlates with dry, firm and separate grains of cooked rice, usually becoming hard after cooling. Intermediate amylose (20–25%) rice is soft but not sticky and generally favoured by most consumers (Juliano 1971). Low amylose cultivars (15–20%) are tender, cohesive, and glossy, and contain nearly all temperate japonica cultivars whereas very low and waxy rice grains are sticky. Rice varieties with similar apparent amylose content, however, can be quite different in their cooking and eating qualities. For example, both Koshihikari and Jasmine rice are highly prized for their unique sensory properties, have the same low amylose content (~17%), but their textures are totally different (Jimenez *et al.* 2010). This happens because rice grain quality is a multidimensional characteristic consisting of many components, and the development of rice quality is associated with other aspects of grain filling and rice kernel development (Huang *et al.* 1998). Further, there may be unknown aspects of starch structure, which can differ between cultivars with the same amylose content. In hexaploid and durum wheat, amylose content ranges from about 18 to 35%,

however, in waxy wheat zero amylose have been produced (Nakamura *et al.* 1995).

Biosynthesis of starch

A number of excellent reviews have covered amylose biosynthesis (Ball *et al.* 1998; Denyer *et al.* 2001), particularly in rice (Vandeputte and Delcour 2004; Jeon *et al.* 2010) and wheat (Singletary 2000; Morell *et al.* 2003; Rahman *et al.* 2005). Thus, we will only summarize the salient points. The synthesis of starch in cereals, require a common set of enzymes, although the importance of the roles and the influence on the starch produced can vary somewhat (Morell *et al.* 2003). During photosynthesis, energy from solar radiation is used for the formation of phosphorylated C3 sugar phosphates and these products are exported from the chloroplasts into the cytosol by a translocator and used to form sucrose, which is transported by the phloem to the starch-storing organs such as endosperm of cereal grains and potato tubers. There, the sucrose is broken down and the products can be utilized through a number of pathways to produce starch (Kammerer *et al.* 1998; Toyota *et al.* 2006). However, in barley and maize, at least there is evidence that the major route for the utilization of glucose involves the reaction of glucose-1-phosphate with ATP outside the amyloplast, to produce ADP-glucose via the enzyme ADP-glucose pyrophosphorylase, although some of this reaction also takes place within the amyloplast (Thorbjørnsen *et al.* 1996). The situation in rice does not appear to be an experimentally tested. Summarizing, the whole biosynthesis of starch in the grain can be arranged in the following three steps (figure 5): (i) $\text{ATP} + \alpha\text{-glucose-1-P} \leftrightarrow \text{ADP-G} + \text{PPi}$; (ii) $\text{ADP-G} + \alpha\text{-1,4-glucose} \rightarrow \text{ADP} + \alpha\text{-1,4-glucosyl-}\alpha\text{-1,4-glucan}$; and (iii) $\alpha\text{-1,4-glucan oligosaccharide} \rightarrow \text{branched } \alpha\text{-1,4-}/\alpha\text{-1,6-glucan}$.

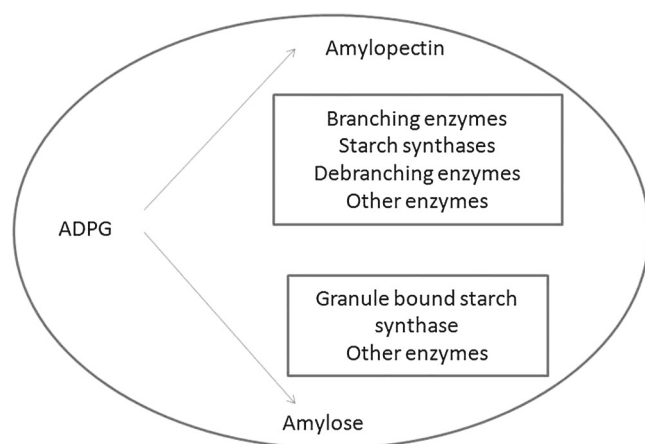


Figure 5. The flow diagram for starch biosynthesis.

The first committed step begins with the conversion of glucose-1-phosphate and ATP to the charged sugar nucleotide, ADP-glucose, and pyrophosphate catalysed by ADP-glucose pyrophosphorylase (AGPase). The enzyme is a key regulator of starch biosynthesis, which performs a high degree of control on the flux of carbon into this pathway (Smith *et al.* 1997; Buléon *et al.* 1998; Tetlow 2006). The expression of AGPase was examined at mRNA and protein levels during seed growth (Reeves *et al.* 1986). In early stages of endosperm formation, the transcript and protein levels are low. They reach the highest level in mid-development and then decline to lower levels by maturation. During grain development, AGPase activity follows a pattern (Ainsworth *et al.* 1995) similar to the rate of starch synthesis (Wardlaw *et al.* 1995; Chanda and Singh 1998).

The sequence of steps in the next phase of starch biosynthesis is not clear. There is also increasing evidence in wheat and maize at least, that the enzymes exist a multimeric complex which may carry out a number of activities simultaneously (Hennen-Bierwagen *et al.* 2008). The composition of such complexes may change during development, influencing their activities. However, for the sake of simplicity it is perhaps better to describe the activities separately and sequentially.

Thus in step (ii), starch synthases (SS) extend regions of α -1,4 glucan through the addition of the glucose residues of ADP glucose to the nonreducing end of a preexisting α -1,4 glucan. Multiple isoforms of SS have been found in higher plants (Marshall *et al.* 1996). One group of SS genes is the granule-bound starch synthases (GBSS), and consists of GBSSI and GBSSII. GBSSI is encoded by the *Waxy* locus in cereals and is essential for amylose biosynthesis (see later). In storage starch, GBSSI catalyses the elongation of amylose (De Fekete *et al.* 1960; Nelson and Rines 1962), while GBSSII is involved in amylose biosynthesis in leaves and other nonstorage tissues (Nakamura *et al.* 1998; Nakamura 2002). Starch granules in wheat pericarp are small

and relatively uniform in size while endosperm starch consists of a population of large and small granules; the amylose to amylopectin ratios appear to differ between starch in the pericarp and the endosperm (Vrinten and Nakamura 2000).

The second group of SS genes (designated SSI, SSII, SSIII and SSIV) is particularly involved in amylopectin synthesis and their distribution within the plastid between the stroma and starch granules differs between species, tissue and developmental stage (Ball and Morell 2003). Their roles in amylose biosynthesis, if any, have not been clarified. These enzymes exist either as soluble forms or as both soluble and minor granule-bound isoforms.

In the conceptually last step (iii), starch branching enzymes (SBEs) catalyse rearrangements of the growing polysaccharide chain by cleavage of internal α -(1,4) linkages and transmit the released reducing ends to a C6 hydroxyl to create new α -(1,6) linkages (Tetlow *et al.* 2008). In addition to SS and SBE, the debranching enzymes (DBEs) also are an important component in the formation of semicrystalline amylopectin, because DBE mutants are often reduced in amylopectin content (Martin and Smith 1995). The rice sugary mutants are evidence of this and it has been shown that the sugary mutation can be complemented transgenically through the introduction of wheat debranching enzyme into the rice sugary mutant (Kubo *et al.* 2005).

The accumulation of RNA transcripts encoding the various enzymes has been described, and the timing of maximum abundance of transcripts relative to grain filling is somewhat different among the different classes of transcript studied (Ohdan *et al.* 2005).

Genetic approaches to altering amylose content

Generally, there are three ways of producing a plant with an altered trait. One is to alter the expression of genes known to be involved in determining the trait and this is most easily done through transgenic approaches. The second approach, through conventional crosses involving lines exhibiting various values of the trait under consideration can alter the expression of many genes at once. This may result in predicted changes but could produce unexpected changes if unsuspected genes are also involved in regulating the trait. The third way, which makes no assumption about the genes involved, requires the isolation of mutants. For amylose biosynthesis, specific genes are known to be involved in regulating the amount but traditional crossing seems to indicate additional unknown factors at work. Further, the isolation of some high amylose mutants in rice, where the genetic basis has not been defined, underscore the gaps in our knowledge. Due to the hexaploid structure of the wheat genome, isolation of mutants by phenotyping is generally not possible and only few mutants have been reported in this way.

Waxy allelic forms and amylose content

The term 'waxy' was first used to name amylose-free mutants of maize, and refers to the waxy appearance of the endosperm of dried kernels, in contrast to the flinty or translucent appearance of normal kernels (Boyer and Hannah 1994). It has been known for a long time that amylose content of rice is predominantly genetically controlled by the *Wx* locus which is located on chromosome 6 (Tsai 1974; Echt and Schwartz 1981; Ainsworth et al. 1983; Sano 1984). The *Wx* gene (which codes for GBSS) is composed of 14 exons and 13 introns (Wang et al. 1990) in rice. In bread wheat, GBSS is encoded by three waxy loci located on the short arm of the chromosomes 7A (*Wx-A1* locus), 7D (*Wx-D1* locus), and on the long arm of the chromosome 4A (*Wx-B1*) (Chao et al. 1989). Wheat lines possessing one or two GBSS null alleles (thus lacking one or two GBSS isoforms), produce starch with reduced amylose content called as a 'partial waxy'. Crossing of these partial waxy mutant lines led to the construction of both completely null waxy lines of hexaploid and tetraploid wheat, and also partial nulls with different combinations of alleles (Nakamura et al. 1995; Urbano et al. 2002). The three *Wx* genes in wheat have different effects on amylose content. The null *Wx-B1b* allele is linked to the largest decline in waxy protein and is associated with the loss of Wx-B1 protein (Miura et al. 1994; Miura and Sugawara 1996) and this allele appears to be important for noodle quality (Araki et al. 1999). In a study by Nakamura et al. (2006), the wheat waxy mutant and wheat lines lacking SSIIa were used as parents in a cross to produce a double mutant line named as 'sweet wheat'. This line accumulated sugars at high levels (Nakamura et al. 2006). Substantial amounts of both maltose and sucrose were present in the developing endosperm of the double mutant, probably due to the impairment of starch biosynthesis.

Wild-type rice, with an active GBSS, contains around 25% (*indica*) or 15% (*japonica*) amylose. The difference is primarily, but not solely, due to differences in the allelic forms of GBSS observed in *indica* (*Wx^a*) or *japonica* (*Wx^b*) rice (Sano 1984). It has been shown that compared with the *Wx^b* allele, the *Wx^a* allele leads to higher level of waxy protein. This is associated with higher amylose content in the grain (Villareal and Juliano 1989; Mikami et al. 2000). The *Wx^a* allele contains G (AGGTATA sequence) at the 5' splice-junction of the intron 1, which causes intron 1 in the *Wx* transcript to be spliced efficiently leading to greater accumulation of mature mRNA. As a result, much more GBSS would be produced and amylose content (AC) in rice endosperm will increase. The *Wx^a* allele is predominant in *O. sativa indica* rice, while the *Wx^b* allele is mainly present in *O. sativa japonica* rice (Sano 1984; Villareal and Juliano 1989; Bligh et al. 1998; Cai et al. 1998; Isshiki et al. 1998; Larkin and Park 1999; Cai et al. 2000).

In a study by Han et al. (2004), the G/T polymorphism explained 80% of the observed AC variation of all 89 nonglutinous rice cultivars tested; all low amylose varieties

tested contain the sequence AGTTATA, while intermediate and high amylose varieties have AGGTATA (Ayres et al. 1997). Although the G/T polymorphism could differentiate low amylose rice varieties, it does not distinguish between intermediate and high amylose varieties (Dobo et al. 2010). Other factors known to be associated with amylose content are polymorphisms in exons 6 and 10 of *Wx* gene (Larkin and Park 2003; Dobo et al. 2010). Overexpression of GBSS by transgenic approaches did not lead to higher amylose in tetraploid wheat (Sestili et al. 2012). In this experiment, a storage protein promoter was used to drive expression of the *Wx-B1* gene and greater amounts of GBSS were produced without an increase of amylose content, demonstrating that the amount of GBSS is not the limiting step in tetraploid wheat. In rice, however, introduction of the *indica* allele (*Wx^a*) using the native promoter into a *japonica* background did lead to a higher amylose content showing in the *japonica* rice tested more glucose can be shifted into amylose with a stronger GBSS activity. This effect plateaued at 40% amylose (Itoh et al. 2003), which was about a quarter higher than in the donor line of the *Wx^a* allele although both the lines had similar Wx protein content. It may be speculated that the increase in the transgenic line was due to the *indica* allele operating in a background of *japonica* alleles, particularly in relation to the SSII allele present in *japonica*. Clearly therefore, although in some genetic backgrounds increase in the Waxy protein can lead to higher amylose content, generally any increase greater than about 40% amylose is not achievable by this route.

Increase of amylose content by impairment of the amylopectin biosynthetic pathway

Impairment of the amylopectin biosynthetic pathway can also increase the proportion of amylose in starch. This is most striking for barley, where the loss of SSIIa led to the production of high amylose (about 60%, Morell et al. 2007). In wheat, the loss of the same enzyme leads to only a minor increase in amylose (Yamamori et al. 2000). Other combinations of impairment of the starch synthases lead to modest increases in amylose in rice (Fujita et al. 2011). Another route to increase amylose content is through the reduction of branching enzyme activity. In the induced amylose extender mutants of rice, starch branching enzyme IIb (SBEIIb) is affected (Satoh et al. 2003) leading to higher amylose content. Transgenic approaches to knockout SBEIIb alone have replicated these effects (Butardo et al. 2011). However, much higher amylose contents have been obtained in rice by targeting SBEIIa, SBEIIb and SBEI, simultaneously (Wei et al. 2010b). In wheat, RNAi knockout of starch branching enzyme IIa genes led to high amylose, firstly in hexaploid wheat (Regina et al. 2006) and then in tetraploid wheat (Lafiandra et al. 2010). Recently, nontransgenic high amylose wheat has been reported through TILLING and crossing single mutants to produce a line with sharply reduced SBEIIa and up to 55% amylose (Slade et al. 2012).

Table 1. QTL analysis of amylose content in rice.

Population/type	Parents	Marker interval	R ²	Allele for the positive effect	Reference
Chromosome 1 <i>O. nivara</i> /Swarna (BC ₂ F ₂)	Wild accessions (IRGC81848 IRGC81832)/rainfed lowland <i>indica</i>	RM243–RM582	5	Swarna	Swamy <i>et al.</i> (2012)
Chromosome 2 Chuan7/Nanyangzhan (F ₈ ; RILs)	<i>indica/japonica</i>	RM525–RM221	2.55%	Nanyangzhan	Lou <i>et al.</i> (2009)
<i>O. nivara</i> /Swarna (BC ₂ F ₂)	Wild accessions (IRGC81848 IRGC81832)/rainfed lowland <i>indica</i>	RM262–RM3515	11%	<i>O. nivara</i>	Swamy <i>et al.</i> (2012)
Chromosome 3 <i>Oryza glaberrima</i> (IRGC 103544)/Caiao (DH) Yuefu/IRAT109	Wild/Upland <i>indica</i>	RM7–RM251	21.5%	Caiao	Aluko <i>et al.</i> (2004)
<i>O. nivara</i> / Swarna (BC ₂ F ₂)	lowland <i>japonica</i> / upland <i>japonica</i>	RM60–C814	25.66%		Guo <i>et al.</i> (2007)
<i>O. nivara</i> / Korean elite cultivar of <i>O. sativa</i> (ILs)	Wild accessions (IRGC81848 IRGC81832)/rainfed lowland <i>indica</i>	RM22–RM7 RM85–RM293	5% 15%	Swarna	Swamy <i>et al.</i> (2012)
Chromosome 5 ZYQ8/JX17 (DH) Taromahalli (TAM) / Khazar (KHZ) (F ₂)	Wild rice/ <i>japonica</i>	RM218–RM554 (2005) RM218–RM554 (2006)	29.4% –		Yuan <i>et al.</i> (2010)
<i>O. nivara</i> / Swarna (BC ₂ F ₂)	<i>indica/japonica</i> <i>indica/indica</i>	RG573–C624 RM480–RM3345	11.8% 11.33%	ZYQ8 Khazar	He <i>et al.</i> (1999) Sabouri (2009)
Chromosome 6 <i>Oryza glaberrima</i> (IRGC 103544)/Caiao (DH) H94/ Zhenzhan 97 (DH) Yuefu/IRAT109	Wild accessions (IRGC81848 IRGC81832)/rainfed lowland <i>indica</i>	RM413–RM13 RM153–RM413	9% 9%	<i>O. nivara</i> Swarna	Swamy <i>et al.</i> (2012)
<i>Oryza glaberrima</i> accession (IRGC : 103544)/V20A (BC ₃ (TC)F ₁) Chuan7/ Nanyangzhan (F ₈ ; RILs)	Wild/upland <i>indica</i> <i>indica/indica</i> Lowland <i>japonica</i> / upland <i>japonica</i> <i>indica/japonica</i>	RM190–RM253 RM190–RM587 MRG5119–C gene C1004–R1962 CDO78–RG653 RM584–RM585 RM588–RM540	73.7% 54.87% 1.10% 19.8% 0.08% 2.84% 7.83%	Caiao Zhenzhan 97 Zhenzhan 97 <i>O. glaberrima</i> Chuan7	Aluko <i>et al.</i> (2004) Fan <i>et al.</i> (2005) Guo <i>et al.</i> (2007) Li <i>et al.</i> (2004) Lou <i>et al.</i> (2009)

Table 1 (contd)

Population/type	Parents	Marker interval	R ²	Allele for the positive effect	Reference
<i>O. rufipogon</i> (IRGC 105491) and IR64 (BC ₂ F ₂)	Upland <i>indica</i> /wild	RM170–RZ398	21.9%	<i>O. rufipogon</i>	Septiningsih et al. (2003)
<i>O. nivara</i> /Swarna (BC ₂ F ₂)	Wild accessions (IRGC81848 IRGC81832)/rainfed lowland <i>indica</i>	RM314–RM3	23%	Swarna	Swamy et al. (2012)
<i>O. rufipogon</i> /Korean elite cultivar of <i>O. sativa</i> (ILs)	Wild rice/ <i>japonica</i>	RM454–RM3567 (2005) RM454–RM3567 (2006)	30.8% 47.7%		Yuan et al. (2010)
Chromosome 7 IR64/Azucena (DH)	Irrigated <i>indica</i> /upland <i>japonica</i>	RG375–RG477	0.06%	IR64	Bao et al. (2002)
<i>O. rufipogon</i> /Korean elite cultivar of <i>O. sativa</i> (ILs)	Wild rice/ <i>japonica</i>	RM298 (2005) RM298 (2006)	30.9% 19.9%	<i>O. rufipogon</i>	Yuan et al. (2010)
Chromosome 8 <i>Oryza glaberrima</i> (IRGC 103544)/Caiaipo (DH) Yuefu/IRAT109	Wild/upland <i>indica</i>	RM230–RM264	10.9%	Caiaipo	Aluko et al. (2004)
Tarommahalli (TAM)/Khazar (KHZ) (F ₂)	Lowland <i>Japonica</i> /upland <i>Japonica indica</i> /indica	R2676–C166	25.66%		Guo et al. (2007)
Asominori/IR24 (RILs)	<i>japonica</i> /indica	RM4955–RM152	20.21%	Khazar	Sabouri (2009)
<i>O. rufipogon</i> /Korean elite cultivar of <i>O. sativa</i> (ILs)	Wild rice/ <i>japonica</i>	RM152–RM8264 G1149–R727 (2000) G1149–R727 (2001) G1149–R727 (2002)	14.45% 17.1% 13.4% 19.2%	Asominori	Wan et al. (2004)
Asominori/IR24 (CSSLs)	<i>japonica</i> /indica	RM230–RM264 (2005) RM230–RM264 (2006)	– 26.7%		Yuan et al. (2010)
Chromosome 9 Asominori/IR24 (RILs)	<i>japonica</i> /indica	RM7356–RM7556 (2008) RM23510–RM23579(2009)	18.91% 17.57–20.72%	Asominori IR24	Li et al. (2011)
Chromosome 11 H94/Zhenshan 97 (DH)	<i>japonica</i> /indica	XNpb36–XNpb103 (2001) C609–C506 (2000) C609–C506 (2001)	8.0% 12.1% 12.5%	Asominori	Wan et al. (2004)
Chromosome 12 H94/ Zhenshan 97 (DH) <i>Oryza glaberrima</i> accession (IRGC: 103544)/V20A (BC ₃ (TC)F ₁)	<i>indica</i> /indica	RM209–RM229	1.85%	H94	Fan et al. (2005)
Tarommahalli (TAM)/Khazar (KHZ) (F ₂)	<i>indica</i> /indica	RM270–RM235 RZ816–RG574	0.85% 0.05%	H94 <i>O. glaberrima</i>	Fan et al. (2005) Li et al. (2004)
Asominori/IR24 (RILs)	<i>japonica</i> /indica	RM276–RM7626	13.33%	Tarommahalli	Sabouri (2009)
		XNpb189-2– XNpb24-2	8.2%	Asominori	Wan et al. (2004)

DH, doubled haploid lines; RIL, recombinant inbred lines; F₂–F₅, populations; BC, backcross population; CSSLs, chromosome segment substitution lines, IL, introgressed lines.

This is lower than observed by (Regina *et al.* 2006) through transgenic technology, which reduced the expression of both SBEII and SBEIIb. The gene for SBEIIa and SBEIIb are closely linked in the proximal region of the long arm of group 2 chromosomes in wheat (Rahman *et al.* 2001) and total loss of activity of both isoforms from all three genomes is therefore a challenging proposition.

QTL mapping and inheritance of amylose content

A purely genetic approach to determine which genes, if any, are involved in determination of amylose content involves mapping studies. Linkage maps are constructed using DNA markers for identifying chromosomal regions that contain genes controlling the traits under study. The whole process of constructing linkage maps and conducting quantitative analysis on the expression of the trait in order to identify genomic regions associated with the trait is known as QTL mapping (McCouch and Doerge 1995). The degree at which QTLs affect amylose content in rice is shown in table 1. QTLs linked to AC have been mapped to 10 chromosomes in at least 12 different populations. Mapping populations were

derived from wild \times *indica* and other type of crosses such as *indica* \times *japonica* rice parents, etc. (table 1). Such studies raise the possibility of introducing unknown beneficial alleles if wild accessions are used in crossing. Transgressive segregants have been recorded previously for AC in populations derived from wild rice accessions (*O. glaberrima*, *O. nivara* and *O. rufipogon*) as donor parents (Septiningsih *et al.* 2003; Aluko *et al.* 2004; Li *et al.* 2004; Yuan *et al.* 2010; Swamy *et al.* 2012). For example, in the study by Swamy *et al.* (2012), 29 families showed an increase of 11% in AC. In another study, 16% of DH lines produced a higher percentage of amylose than the high amylose producing *O. glaberrima* parent (Aluko *et al.* 2004). There may be still other QTLs that are equally important but are not highlighted in this study, mainly because of the lack of allelic differences in the parents used. In rice, the marker interval RM190–RM253 on chromosome 6 (with reference to the genetic map derived from *Oryza glaberrima*/Caiapo DH lines; Aluko *et al.* 2004) has frequently been associated with AC (figure 6). Many QTLs for AC were identified in this region (He *et al.* 1999; Tan *et al.* 1999; Septiningsih *et al.* 2003). Colocation of QTLs for gel consistency and gelatinization temperature were also observed on this region

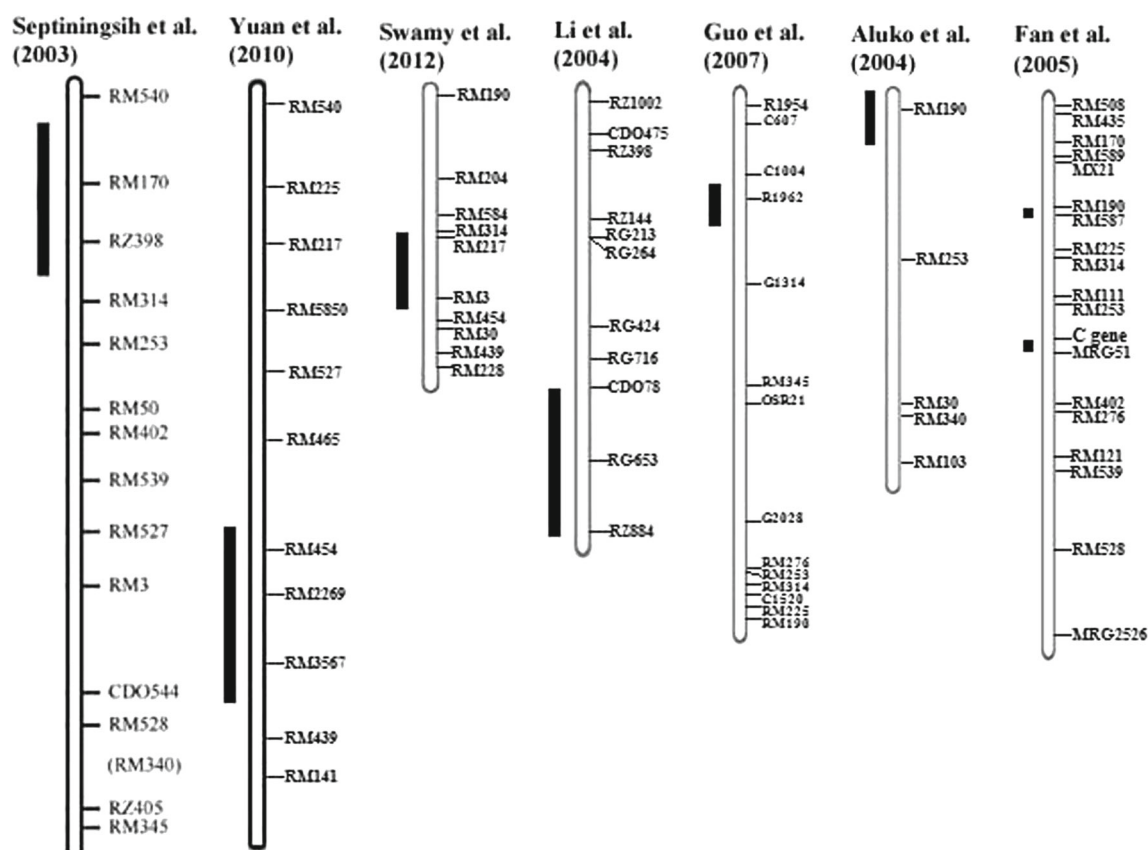


Figure 6. QTLs mapped for amylose content in rice chromosome 6 in different populations. Parts of rice chromosome 6 are shown as vertical bars with mapped markers indicated to the right. Filled black boxes indicate flanked markers for QTL position.

(Tan et al. 1999; Fan et al. 2005; Yuan et al. 2010). This segment contains the *Wx* gene and the locus explained variation in AC. The extent of AC variation accounted for varied depending on the crosses examined varying from 2.84 to 91.1% variability in the dataset across several mapping populations (Ayres et al. 1997; He et al. 1999; Aluko et al. 2004; Fan et al. 2005), respectively. Clearly, the proportion explained will depend on the allelic status of the *Wx* gene in parents used in the cross.

QTLs related to alleles of the *Wx* gene have also been reported in wheat. Using 98 single-chromosome recombinant substitution lines derived from a cross of Chinese Spring and Chinese Spring (Kanto107 4A) with a low amylose content due to the null *Wx-B1b* allele, it was found that the presence of the null allele accounted for 70% of the variation in amylose content. However, an additional QTL (*QAmc.ocs-4A.1*) was identified with minor effect (17%) on amylose content. This was mapped in the 6.2-cM *Xbcd1738/Xcdo1387* interval on the short arm of chromosome 4A (Araki et al. 1999) and the allele from Kanto107 led to an increase in amylose content with an additive effect of 0.3%. The same map position was shared by two QTLs for peak viscosity ($r^2 = 0.24$) and breakdown viscosity ($r^2 = 0.26$), which explained 24 and 26% of the variation, respectively (Araki et al. 2000). Colocation of QTLs for plant height, heading time and flour swelling volume was also identified at, or closely linked with *Wx-B1* locus on chromosome 4A (Miura and Sugawara 1996; Araki et al. 1999).

QTLs controlling AC in rice were identified on chromosomes other than chromosome 6 such as chromosomes 1, 2, 3, 5, 7, 8, 9, 11 and 12 (table 1). These results support the idea that both the major *waxy* gene and modifying genes control AC, as previously reported by McKenzie and Rutger (1983) and He et al. (1999). In addition, conventional crosses with wild relatives can introduce alleles of genes that influence amylose content in unexpected ways and lead to transgressive segregation (Fasahat et al. 2012b). In wheat too, there is evidence that the *Wx* genes are not the only genes that control amylose content. In the study by Sun et al. (2008), two QTLs, *QAlc.sdau-2A* and *QAlc.sdau-2D*, with 12.57 and 13.29% phenotypic variation, respectively were identified on group 2 chromosomes in a set of 131 RILs derived from a winter wheat cross (Chuan 35050 × Shannong 483). Transgressive inheritance was also recorded.

High amylose mutants with unexplained lesions

The rice mutant Goami represents a mutant with higher amylose but one where the molecular lesion remains unexplained (Butardo et al. 2012). Similarly, the RS111 line described by Yang et al. (2006) represents a phenotype with elevated amylose with intriguingly altered properties; its genetic basis does not appear to have been reported.

Conclusion

Starch is a critical part of the human diet and the knowledge of starch synthesis in the cereal endosperm has improved greatly in the last few years. The biosynthetic pathway from sucrose to the starch granule is complex. The absence of amylose does not prevent formation of starch granules and if the amylose make up more than about 50% of the total starch then abnormal morphologies can result. Alterations of the proportion of amylose in starch can influence both the palatability of the grain and its health impact. The principal gene controlling amylose content is the *Waxy* (*Wx*) gene but multiple QTLs have been detected in rice and wheat, which indicate that AC is also influenced by other genes. Further analysis of amylose structure and biosynthesis is warranted to gain greater appreciation of the link between structure and functionality. This will also provide strategies to breed grains with desired properties.

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