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Different isoforms of starch-synthesizing enzymes controlling amylose and amylopectin content in rice (*Oryza sativa* L.)

Manish K. Pandey [a,b,*], N. Shobha Rani [a], M. Sheshu Madhav [a], R.M. Sundaram [a], G.S. Varaprasad [a], A.K.P. Sivaranjani [a], Abhishek Bohra [b], G. Ram Kumar [a], Anirudh Kumar [a]

[a] Crop Improvement Section, Directorate of Rice Research (DRR), Rajendranagar, Hyderabad 500030, India
[b] Department of Genetics, Osmania University, Hyderabad 500007, India

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ABSTRACT

Starch, composed of amylose and amylopectin, greatly influences rice cooking and textural quality, which in turn is controlled by various isoforms of several enzymes. Activity of one or more isoforms of starch-synthesizing enzymes results in various forms of starch structure based on the amylopectin chain length and average external, internal and core chain length distribution and hence results in varying physicochemical and cooking quality. Since the synthesis of starch is highly complex, it is crucial but essential to understand its biosynthetic pathway, starch structure and effects on the physicochemical properties that control eating and cooking quality, and alongside conduct research on gene/QTL mapping for use in marker-assisted selection (MAS) with a view to improve and select cultivars with most desirable range and class of rice starch properties. This article presents the updates on current understanding of the coordination among various enzymes/isoforms towards rice starch synthesis in endosperm and their effect on rice grain physicochemical, cooking and eating qualities. The efforts in identifying regions responsible for these enzymes by mapping the gene/QTLs have provided a glimpse on their association with physicochemical and cooking properties of rice and, hence, improvement is possible by modifying the allelic pattern, resulting in down or nil regulation of a particular enzyme. The clear understanding of the tissue specific coordination between enzyme isoforms and their subsequent effect in controlling eating and cooking properties will enhance the chances to manipulate them for getting desired range of amylose content (AC) and gelatinization temperature (GT) in improved cultivars through combining desired alleles through MAS.

Contents

* Corresponding author at: Applied Genomics Laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India and Department of Plant Pathology, University of Georgia (UGA)/USDA-ARS, Tifton, GA 31793, USA.
E-mail addresses: m.pandey@cgiar.org, m.pandey@uga.edu (M.K. Pandey).

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1. Introduction

Starch, an end product of photosynthesis in source tissues, is stored as energy reserves in the sink tissues and is composed of two major components, i.e., amylose and amylopectin. Earlier studies established strong association of starch structure with physical behavior and functionality (Fujita et al., 2003; Nakamura et al., 2002; Preiss and Sivak, 1996; Tetlow et al., 2004; Zhang et al., 2011). The variations in starch structure arise due to differential expression of various isoforms of starch biosynthetic enzymes. The varietal differences in the amylopectin structure exist predominantly due to chain length variation and play a critical role in determining physicochemical properties of starch in rice endosperm. Amylose content (AC) and gelatinization temperature (GT) are the two main measures to assess the rice grain quality. AC determines the firmness and sticky nature of cooked rice while rice with high GT requires higher temperature, more water and time to cook than those with low or intermediate GT. As the GT is directly correlated to the time required to cook rice, therefore, rices with intermediate GT are preferred over those with high or low-GT. These two properties have highest effect on cooked rice grain quality and thus play major role in influencing consumer's preference. In several studies, both AC and GT were found highly associated with eating and cooking properties of rice (Juliano et al., 1964; Kaw and Cruz, 1990; Shobha Rani et al., 2011a; Tang et al., 1989) and hence are important traits to consider together for improving rice grain quality of high yielding rice varieties worldwide to meet consumer's preference. Rice with good grain quality fetches higher returns to the farmers beside high demand due to increasing population/consumers (Shobha Rani et al., 2006). Therefore, it is imperative to improve AC and GT in desirable range into conventionally bred varieties as well as in the hybrids for their better acceptance by farmers, traders and consumers.

Recent studies have improved current understandings about regulating network of several isoforms of various enzymes for starch biosynthesis in higher plants. The structural variation, which contributes greatly to the physicochemical properties, is thought to be caused by the differences in the composition and relative activities of the isozymes of starch synthase (SS), starch branching enzyme (SBE) and debranching enzymes (DBE). Although few efforts were made to highlight the importance of rice grain quality and their possible improvement to meet consumer's preference (Shobha Rani et al., 2006), regulation of starch synthesis metabolism in higher plants and role/genetics of different enzymes in starch biosynthesis (James et al., 2003; Jeon et al., 2010; Keeling and Myres, 2010; Tetlow et al., 2004; Tian et al., 2010; Vandeputte and Decour, 2004) and identification/mapping of gene(s)/QTLs for AC and GT in rice (Shobha Rani et al., 2008), none of these studies emphasized to understand the association of different isoforms of starch-synthesizing enzymes with rice grain quality traits either directly or indirectly. However, the present article addresses the recent developments in biochemistry, genetics and genomics in relation to various enzymes controlling starch synthesis and their effect on the physicochemical and cooking properties of rice. We review recent speedy developments in all these individual areas which improved our existing understanding on enzyme coordination for starch synthesis, genes involved and identification of genomic regions responsible for grain quality traits. Finally, we suggest for a multidisciplinary holistic approach to get desirable range of physicochemical and cooking properties in rice cultivars through manipulating enzyme specific genes.

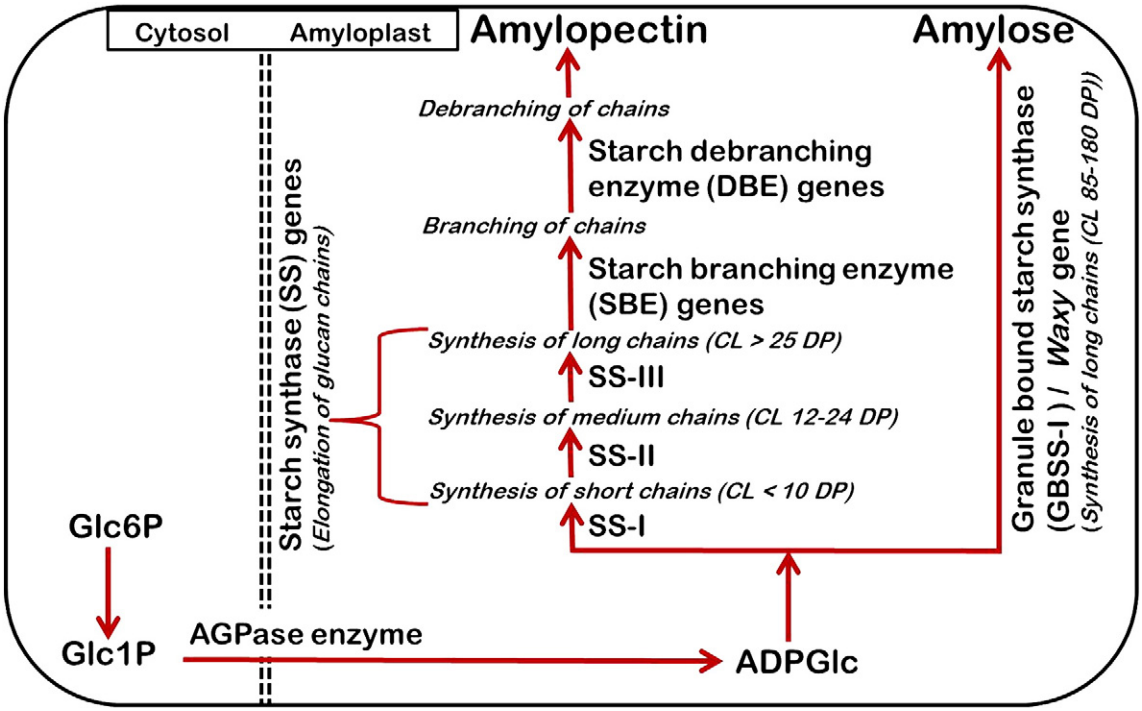

Fig. 1. A diagrammatic presentation of coordination between different starch-synthesizing genes in rice endosperm. This illustration shows flow of biochemical activity leading to synthesis of amylose and amylopectin along with role of different starch-synthesizing genes at each step.

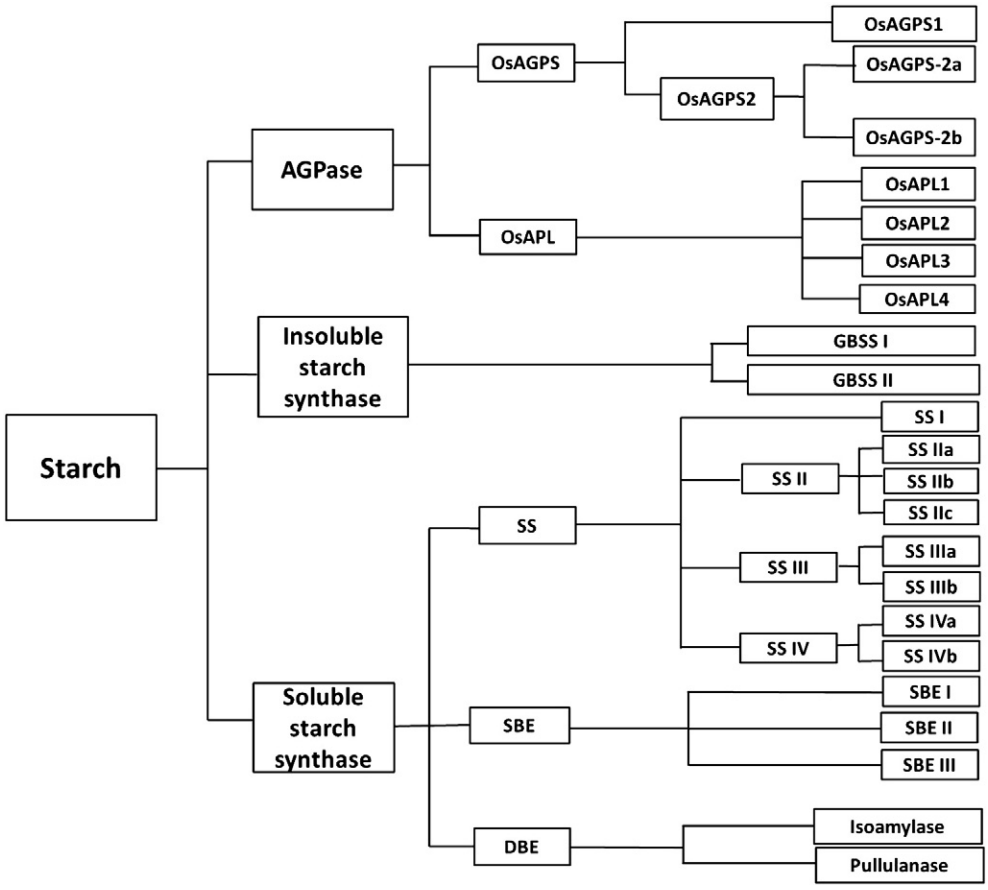

Fig. 2. The organogram showing the various isoforms of starch-synthesizing enzymes involved in starch synthesis in rice. AGPase: adenosine diphosphate glucose pyrophosphorylase; SS: soluble starch synthase; SBE: starch branching enzyme; DBE: debranching enzyme.

2. Starch-synthesizing enzymes and their isoforms in rice

Starch biosynthesis in higher plants including rice is catalyzed by four classes of enzymes, namely, ADP-Glc pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzymes (SBE) and starch debranching enzymes (DBE). Of these, granule bound starch synthase-I (GBSS-I) (insoluble starch synthase) enzyme controls the synthesis of amylose in rice endosperm, while the soluble starch synthase (SS), SBE and DBE together control synthesis of amylopectin (Bao et al., 2002; Myers et al., 2000; Nakamura, 2002; Smith et al., 1974; Tanaka et al., 2004; Tetlow et al., 2004; Zhang et al., 2011) (Fig. 1). Several studies lead to identification of various SS isoforms through gene sequencing in several plant genomes. In rice, there are 10 SS isoforms which can be grouped into five types, namely, GBSS (I, II), SSI, SSII (SSIIa, SSIIb and SSIIc), SSIII (SSIIIa and SSIIIb) and SSIV (SSIVa and SSIVb) isoforms (Hirose and Terao, 2004; Tetlow et al., 2004; Zhang et al., 2011) (Fig. 2). All these isoforms of starch-synthesizing enzymes coordinate together and form a regulating network to control starch synthesis in rice endosperm which affects grain cooking and eating quality (James et al., 2003; Jeon et al., 2010; Tian et al., 2010). Hence, the challenge lies in understanding the genetic basis and identification of genomic regions controlling these isoforms in order to develop cultivars with desirable combination of alleles controlling these isoforms.

3. Physicochemical traits affecting rice grain quality

Rice grain quality is mainly defined by four constituents, namely, milling, appearance, cooking and nutritional quality. Of these four constituents, traders are more interested in first two while consumers are more concerned for later two constituents. Furthermore, the consumer preferences for grain quality vary in each region across the world based on their food habits. Because of this reason, improvement in rice grain cooking quality has become most important research component in almost all the rice improvement programmes worldwide. Traits such as amylose content (AC) and gelatinization temperature (GT) which exert major effect on the eating and cooking qualities are itself controlled by physicochemical properties of starch in endosperm of rice grains.

Table 1
Inheritance of the starch properties traits in the rice.

Amylose content		
1	Monogenic with modifiers (incompletely dominant)	Seetharaman (1959); Kahlon (1965); Bollich and Webb (1973); Somrith (1974); Chang and Li (1981); Chauhan and Nanda (1983)
2	Two complimentary genes	Stansel (1966)
3	Digenic with partial dominance of high over low	Heda and Reddy (1986)
4	Dosage effect of genes	Kumar and Khush (1986); Heu and Park (1976); Okuno and Yano (1984)
Gelatinization temperature		
1	Two pairs of major genes	Tomar and Nanda (1985)
2	Monogenic recessive	Heu and Choi (1973), McKenzie and Rutger, 1983; Umemoto et al., 2002; Gao et al., 2003
3	Dominant and additive effects	Hsieh and Wang (1988)
4	Additive gene effects	Somrith (1974)
5	Trigenic	Stansel (1966)
6	Polygenic, additive and non-additive	Puri and Siddiq (1980)

Table 2
Reported QTLs for the starch property traits in the rice.

S. no.	Locus	Chrom.	Marker interval/marker	PVE (%)	References
Amylose content					
1.	*Wx*	6	Wx	91.1	He et al., 1999
	qAC5	5	RG 573-C624	11.8	
2.	–	6	*Waxy*-RM 204	58.69	Lanceras et al., 2000
	–	4	G177A-GA7-2	15.99	
	–	3	RM81-C158	11.28	
	–	7	OSR22-RM 10	9.18	
3.	*qAC-6*	6	R 2869-R 1962	80.7	Li et al., 2003
	qAC-5	4	C 1100-R 1783	2.35	
	qAC-4	5	C 624- C 128	1.45	
	qAC-3	3	R 1927-R 3226	1.6	
4.	*wx*	6	RM 170	28.2	Septiningsih et al., 2003
5.	*amy 6*	6	RM190-RM253	73.3	Aluko et al., 2004
	amy 3	3	RM 7RM 251	-	
	amy 8	8	RM230-RM264	-	
6.	*qAC-8*	8	G1149-R727	16.5	Wan et al., 2004
	qAC-9B	9	C609-C506	12.3	
	qAC-12	12	XNpb189-2-XNpb24-2	14.7	
7.	*qAC-6*	6	RM190-RM510	61.8	Tian et al., 2005
8.	*qAC-2*	2	R1843-G132	5.83	Sun et al., 2006
	qAC-6	6	S1084-R1952	74.67	
9.	*amy6-1*	6	RM3-RM217	39.6	Amaravathi et al., 2008
10.	–	2	RM183-RM573	1.1	Wang et al., 2007
	–	6	RM586-MX21	88	
	–	9	RM296-RM105	1.2	
11.	*qAC-1-1*	1	R753-G359	-	Zheng et al., 2008
	qAC-1-2	1	C904-R2632	-	
	qAC-4-3	4	C56-C820	-	
	qAC-6-4	6	C952-waxy	55.8	
12.	*qAC-6*	6	RM204-RM276	9.0	Shobha Rani et al., 2011b
Gelatinization temperature (GT)					
1.	*alk*	6	CT506-C235	82.4	He et al., 1999
	qASS-6	6	CT201-RZ450	24.6	
2.	–	6	C1478-RZ667	60.3	Lanceras et al., 2000
	–	2	RG73-RM6	12.2	
	–	6	RM3-RM238	8.57	
3.	*qASS-6a*	6	G200-C1478	69.44	Li et al., 2003
	qASS-6b	6	R2869-R1962	8.1	
	qASS-3	3	C25-C515	2.32	
	alk	6	RM50-RM527	-	Gao et al., 2003
4.	*alk6-1*	6	RM190-RM253	50.1	Aluko et al., 2004
	alk6-2	6	RM253-RM162	44	
5.	*qGT-1*	1	C955-C970	13.8	Wan et al., 2004
	qGT-3	3	C1677-R3156	20.9	
6.	*qGT-6*	6	RM276-RM121	80.3	Tian et al., 2005
	qGT 3-1	3	R2856-R3226	8.31	Sun et al., 2006
7.	*qGT-6*	6	G200-R2171	64.42	
	asv6-1	6	RM3-RM217	6.9	Amaravathi et al., 2008
8.	qGC-3	3	R2856-R3226	12.74	
9.	–	6	MX21-RM204	7.7	Wang et al., 2007
	–	6	RM276-RM549	87.8	
10.	qASV-6-1	6	Waxy-C1496	11.3	Zheng et al., 2008
11.	qGT-2	2	RG256-RZ213	14.41	Govindraj et al., 2009
	qGT-5	5	RZ70-RZ225	15.39	
12.	*qGT-6*	6	RM276-RM217	30.7	Shobha Rani et al., 2011b

3.1. Amylose content (AC)

Starch has two major components, namely, linear ά -polyglucan amylose and branched ά -polyglucan amylopectin. In general, stored starch in the higher plants is composed of 20–30% amylose and 70–80% amylopectin. Rice varieties can be classified based on varied range of AC such as waxy (0–2%), very low (3–12%), low (13–20%), intermediate (21–25%) and high (≥26%) (Juliano et al., 1981). Rice grains with high AC (≥26%) cook dry, become less tender and hard upon cooling while rice grains with low AC (<20%) cook moist and become very sticky. That is why rice with intermediate AC (20–25%) is preferred in majority of the rice-growing/consuming regions of the world, except in few regions where only low-AC *japonicas* are preferred. Therefore, the efforts need to be intensified in developing perfect markers for differentiating each class of AC and marker-assisted introgression of desired allele, which will help the breeders in developing varieties with preferred AC range as per local and international consumer preference for rice cooking quality.

3.1.1. Genetics and mapping of quantitative trait loci (QTLs) for AC

Several studies on the genetics of AC had reported involvement of one major gene and several modifiers with high AC incompletely dominant over low AC (Chang and Li, 1981; Chauhan and Nanda, 1983; Somrith, 1974) (Table 1). However, in addition to the *waxy* gene which controls AC, the involvements of two complimentary genes were also reported (Stansel, 1966). Furthermore, the differences in AC were observed due to the dosage of amylose genes in the endosperm (Kumar and Khush, 1986), but the AC was not directly proportional to the number of W_x dose (Heu and Park, 1976; Okuno, 1978). There are several reports on the association of these markers

Table 3
The primer sequences of the SSR, CAPS, STS and SNP markers identified for the starch-synthesizing genes in rice.

Genes	Type of marker	Forward primer (5–3′)	Reverse primer (5–3′)	References
Wx	SSR	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	Ayres et al., 1997
	CAPS, *ACC*1	TTTCCAGCCCAACACCTTAC	TTGCAGATGTTCTTCCTGAT	
Sss1	SSR	GATCCGTTTTTGCTGTGCCC	CCTCCTCTCCGCCGATCCTG	Bao et al., 2002; Larkin and Park, 2003
	STS	TCTAGATTGCTACACGTGAGAGG	TCTCCACGATAACTTCCACC	Ayres et al., 1997
Sss11a (alk)	STS	TCTAGATTGCTACACGTGAGAGG	GGAGCCACCTGTAAAGCGTG	
	SNP (1)	CTGGATCACTTCAAGCTGTACGAC	GCCGGCCGTGCAGATCTTAAC	Bao et al., 2006
	SNP (2)	CAAGGAGAGCTGGAGGGGGC	ACATGCCGCGCACCTGGAAA	
	SSR	CTTTGATAGTTCGAATGGTT	CAATGTTTCTCCGTGATGAT	Gao et al., 2003
Sbe I	SSR	ATTTCTTTGGCCACAGGCGA	CCCAGATTCGGAACAAGAAC	Akagi et al., 1996
	STS	GAGTTGAGTTGCGTCAGATC	AATGAGGTTGCTTGCTGCTG	Han et al., 2004
	CAPS,*ACC*11	CCGAGGGAATGCCAGGAGTACCAG	GAACCACAACCAAGTCCAAGGCAA	Liu et al., 2006
	STS	GAGTTGAGTTGCGTCAGATC	CAGCAGCAAGCAACCTCATT	
Sbe III	CAPS, *Spe*1	GTCTTGGACTCAGATGCTGGACTC	ATGTATAACTGGCAGTTCGAACGG	
	STS	TCGGTCAATTCGGTTAGTCTCCTC	ACATCCTCTAGCATACTGGCGACTC	
Pul	STS	GGGTTCGCTTTCACAACACAG	GTCACGACATAAGAGAAGCTGC	Ayres et al., 1997
	STS	AGTTCGCTAGTCATCTGCTCG	CCACATGTCCTTGTCTCCACTT	
Isa	CAPS, *Ssp*1	CTTGTGTCTGGTGAGGCTTCTA	CCTAGTGACTGGACTCATGGTT	
	STS	CCTGTCTTGCACGTGCGGTA	GCACGGTTCTGATGTACGAGAG	

with different quality characteristics in the rice germplasm. Among these, two SNPs in the exons of *Wx* gene that resulted in amino acid substitutions were found to be associated with AC and viscosity characteristics (Larkin and Park, 2003). However, with indel markers for *SBE1, SBE3* and *DBE* genes, no association could be established between the marker alleles and the variation in apparent amylose content (AAC) and paste viscosity characteristics in a segregating population. More significantly, efforts made in the mapping of QTLs yielded in identification of one major QTL on chromosome 6 with high phenotypic variation along with several small effect QTLs distributed on other regions of the rice genome (He et al., 1999; Lanceras et al., 2000; Lee et al., 2007; Gao et al., 2003; Septiningsih et al., 2003; Aluko et al., 2004; Wan et al., 2004; Tian et al., 2005; Sun et al., 2006; Amaravathi et al., 2008; Wang et al., 2007; Zheng et al., 2008; Govindraj et al., 2009; Shobha Rani et al., 2011b) (Table 2). Association study with above identified flanking markers revealed that these molecular markers can differentiate low-AC genotypes from the high and intermediate AC genotypes; however, none of the identified linked markers can differentiate genotypes with intermediate and high AC.

3.1.2. Identification of allele-specific markers

Amylose is a linear polymer of ά-(1–4) linked D-glucopyranosyl units with few α-(1–6) linkages and has average degree of polymerization (DPn) of 800–4920, average chain length (CL) of 250–670 and β-amylolysis limits of 73–95% (Morrison and Karkalas, 1990). It is controlled by *waxy* gene encoding granule bound starch synthase-I (GBSS-I). The simple sequence repeat (SSR) polymorphism with respect to $(CT)_n$ repeats was also correlated (Bligh et al., 1995; Tan and Zhang, 2001) for *waxy*, low, intermediate and high AC with $(CT)_{17}$, $(CT)_{18–19}$, $(CT)_{11–10}$ and $(CT)_{11–10,}$ respectively. Few studies (Isshiki et al., 2000; Chen et al., 2008; Mikami et al., 2008) confirmed the existence of five alleles at *waxy* locus, i.e., *waxy*, Wx^a, Wx^{in}, Wx^b and Wx^{op}. A single nucleotide polymorphism (SNP) in intron 1 differentiates low amylose (Wx^a) from the high (Wx^b) and intermediate (Wx^{in}) classes. Further, a SNP at exon 6 (which changes serine to tyrosine) can differentiate intermediate AC from the high AC while SNP at exon 4 (which changes aspartate to glycine) shows low GBSS activity and gives opaque (Wx^{op}) phenotype. As the SNP genotyping facility is not available in majority of the breeding institutes, allele/SNP specific PCR-based markers may be developed to differentiate various AC classes and should be integrated with conventional breeding programme for grain quality improvement. It is also estimated that the *Wx* locus alone may not explain all the observed AC variation among rice cultivars and, hence, some minor genes might also be involved. Nevertheless, until availability of robust and perfect markers for each AC class, *Waxy* SSR, CT-SSRs together with G-T SNP may be used by rice breeders to develop rice varieties with desirable range of AC (Table 3).

3.2. Gelatinization temperature

Gelatinization temperature (GT) is a physical property of the rice starch and refers to the range of temperature within which starch

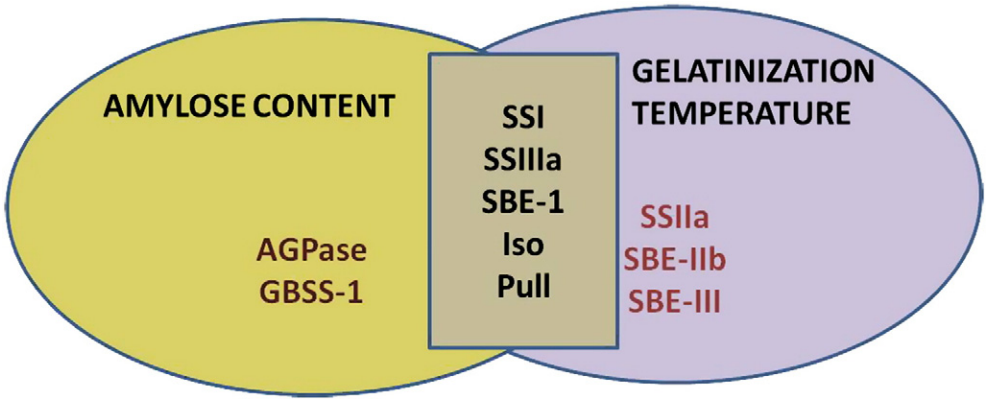

Fig. 3. Coordination between AGPase, GBSS-1, soluble starch synthase (SS-I, SS-IIb, SS-IIc, SS-IIIa), branching enzymes (SBE-I, SBE-IIb, SBE-IIIb) and debranching enzymes (iso, pull) in synthesizing starch in rice endosperm, controlling the two key rice grain quality traits, i.e., amylose content and gelatinization temperature.

Table 4
Enzymes playing key roles in the starch biosynthesis in the rice.

Enzymes	Role in starch biosynthesis	Reference
ADP-glucose pyrophosphorylase		
ADPglc PP	Transport of ADP-glucose	Asaoka et al., 1993
OsAPS-1	Starch biosynthesis in seed	
OsAPS-2a	Starch biosynthesis in leaves	Lee et al., 2007
OsAPS-2b	Starch biosynthesis in seed	
OsAPL-1, 3,4	Starch biosynthesis in plastid	
OsAPL-2	Starch biosynthesis in seed	
Granule bound starch synthase enzyme (GBSS)		
GBSS-I	Encoded by the *Waxy* locus facilitating amylosyntheses in storage tissues through formation of long chains. It was also found responsible for extension of long glucans within the amylopectin fraction.	Sano, 1984; Yano et al., 1988; Asaoka et al., 1993; Mikami et al., 1999; Tetlow et al., 2004
	Extension of long glucan chains within amylopectin fractions	Delrue et al., 1992; Maddelein et al., 1994; van de Wal et al., 1998
GBSS-II	Amylose synthesis in leaves and other non-storage tissue of plants	Nakamura, 2002; Fujita and Taira, 1998; Vrinten and Nakamura, 2002
Starch synthases (SS)		
SS-I	Synthesis of short chains of DP 8–12 from very short DP 6–7 chains emerging from the branch point in the A or B (1) chain of amylopectin. Functions from the very early to the very late stage of endosperm development.	Nakamura, 2002; Umemoto et al., 2002; Fujita et al., 2006; Nakamura, 2002; Umemoto et al., 2002; Nakamura et al., 2005; Zhang et al., 2011
SS-IIa	Synthesis of medium size chains (DP 12–24) by elongating A and B1 chains of amylopectin. Its activity determines type (*indica* or *japonica*) of amylopectin structure in rice endosperm.	
SS-IIb	Barely expressed in the seed and only active earliest period of grain formation. Mostly expressed in leaves.	Ohdan et al., 2005
SS-IIc	Immerged much before SS-IIa & b and is expressed mostly in endosperm along with SS-IIa.	Ohdan et al., 2005
SS-IIIa	Formation of long B1 and B2 chains of amylopectin in endosperm	Nakamura, 2002; Ohdan et al., 2005; Fujita et al., 2007; Zhang et al., 2011
SS-IIIb	Mostly expressed in leaves and less in seed.	Ohdan et al., 2005
SS-IIIc and SS-IVa & SSIVb	Function unknown	
Starch branching enzymes (SBE)		
SBE-I	Synthesis of B1, B2, B3 chains of amylopectin	Nakamura, 2002; Satoh et al., 2003a, 2003b
SBE-IIa	Starch synthesis in leaves and other non-storage tissue of plants	Blauth et al., 2001; Nakamura et al., 2010
SBE-IIb	Role in synthesis of A and B1 chains of amylopectin in storage tissues by transferring short chains which are then extended by SS enzymes to form A and B1 chains of amylopectin	Asaoka et al., 1993; Nishi et al., 2001; Tanaka et al., 2004; Nakamura et al., 2010; Butardo et al., 2011
SBE-III	Synthesis of 1–6 branching linkage	Chen et al., 2004; Nakamura et al., 2010
Starch debranching enzymes (DBE)		
DBE (isoamylase and pullulanase)	Debranching amylopectin chains by hydrolyzing ά (1–6) bonds	Nakamura, 1996; Kubo et al., 1999; Fujita et al., 2003; Wong et al., 2003; Utsumi et al., 2011

granules start swelling irreversibly in hot water. In other words, GT determines the time taken to cook the rice. The quality and quantity of starch in rice endosperm together with GT strongly influence the cooking quality of rice (Ghosh and Govindswamy, 1997) such as water uptake, volume expansion and linear kernel elongation (Tomar and Nanda, 1985). The rice varieties with intermediate GT are preferred and mostly the Indian basmati varieties have intermediate GT but these varieties are poor in yield.

3.2.1. Genetics and mapping of quantitative trait loci (QTL) for GT

Genetical studies could not conclude the inheritance pattern due to several contradictory reports such as dominant and additive (Hsieh and Wang, 1988), digenic (Stansel, 1966), and polygenic (Puri and Siddiq, 1980) mode of inheritance. However, several QTL mapping studies zeroed on existence of a major QTL on chromosome 6, explaining high phenotypic variation (Aluko et al., 2004; Amaravathi et al., 2008; Gao et al., 2003; Govindraj et al., 2009; He et al., 1999; Lanceras et al., 2000; Shobha Rani et al., 2011b; Sun et al., 2006; Tian et al., 2005; Wan et al., 2004; Wang et al., 2007; Zheng et al., 2008) (Table 2). These studies also pointed out the possible role of either alkali degeneration gene (*alk*) (Aluko et al., 2004; Bao et al., 2004; Fan et al., 2005; Gao et al., 2003; He et al., 1999) or *waxy* gene (Tan et al., 1999; Zheng et al., 2008) also in controlling GT. More interestingly, the starch synthase IIa (*SSIIa*) gene was found to be located at the *alk* locus on chromosome 6 in the rice genome (Umemoto et al., 2002). Map-based cloning of the *alk* locus revealed that this locus encodes *SSIIa* and nucleotide substitutions in the coding sequence of *SSIIa* reported to cause the alteration in GT (Gao et al., 2003).

3.2.2. Role of amylopectin structure in controlling the GT

The gelatinization behavior of starch granules in rice is predominantly determined by the amylopectin structure (Lanceras et al., 2000; Umemoto et al., 2002) and amylopectin consist of ά (1–4) linked D-glucosyl chains and is highly branched with α (1–6) bonds (Bulean et al., 1998). Rice starch amylopectins have chain length (CL) of 19–23 (Takeda et al., 1987). Compared to amylopectins of *Japonica* (CL 19–20), *Indica* have higher CL of 21–22, while waxy *Japonica* rice starches have the lowest CL (17–19) (Morrison and Karkalas, 1990). Very short chains were found negatively correlated, while the longer chains were found positively correlated to GT (Nakamura et al., 2002; Noda et al., 2003; Vandeputte and Decour, 2004). The mechanism underlying the synthesis of amylopectin, which controls the GT, is highly complicated. Three classes of enzymes are involved in the synthesis of amylopectin and each class has multiple isoforms. Synthesis of amylopectin, a complex structure, arises due to subtle interplay between starch synthase (SS), starch branching enzyme (SBE) and the starch debranching enzyme (DBE). The function of the SS is to catalyze the chain-elongation reaction of α-1-4-glucosidic linkage by transferring a glucose moiety from ADP-glucose to the non reducing end of the linkage; the SBE introduces a α-1-6-glucosidic linkage into a polyglucan and the DBE, which hydrolyzes an α-1-6 glucosidic linkage of a-polyglucan (Myers et al., 2000; Nakamura, 2002). The above studies indicates clearly that group of SS enzymes (SS-I, SS-II, SS-III and SS-IV), SBE and DBE play important role

in the amylopectin biosynthesis, structure and their distribution in different tissues (Figs. 1–3). These structural changes in the amylopectin increase/decrease the gelatinization temperature of endosperm starch.

3.2.3. Identification of allele-specific markers

Since various isoforms of several enzymes are involved in the synthesis of amylopectin, it is very complicated but necessary to understand the contribution of each isoform of all the enzymes in isolation and in combinations. The ultimate requirement is the development of isoform-specific molecular markers for possible improvement through MAS using allele-specific markers. To achieve this, molecular markers tagged to genes encoding two soluble SSs (SSSI and SSSIIa), two SBEs (SBEI and SBEIII), and two starch DBEs (isoamylase and pullulanase) enzymes have been identified and evaluated for the effects of these enzymes on the cooking quality (He et al., 2006) (Fig. 3). In parallel, Nakamura et al. (2005) analyzed the effects of amino acid replacement caused by these SNPs on the enzyme activity and on the amylopectin structure and GT, and the results indicated that two SNPs (at 4,198 and 4,229/4,330 bp) are essential for *SSIIa* activity and granule association. In addition, nucleotide diversity of *SSIIa* gene was studied to investigate the relationships between the SNPs identified in these rices and their GT values. This study confirmed role of GC/TT polymorphism in *SSIIa* in differentiating the rices with high- or intermediate-GT from those with low-GT with about 90% correct prediction (Bao et al., 2006). In another study, at least 3 different alleles for *SSIIa* gene were postulated, i.e., one in the *Japonica* rice and two in the *Indica* rice varieties. Furthermore, two alleles for either low- or high-GT for *alk2(t)* gene have also been reported. Thus, the rich diversity of the GT character in the rice probably resulted from the various combinations of these alleles. However, still further study is required to quantify the effects of individual as well effects of many enzymes in combination using functional genomics approach to move towards pyramiding of desired allele combinations.

4. Regulating network of enzymes for starch biosynthesis in rice

Starch biosynthesis in higher plants is catalyzed by four classes of enzymes, namely, ADP-Glc pyrophosphorylase (AGPase), SS, SBE and DBE (Myers et al., 2000; Nakamura, 2002; Smith et al., 1974; Tetlow et al., 2004; Zhang et al., 2011) (Figs. 1–3; Table 4). Recent advancements in the research have improved our understanding on the structure and functions of various isoforms of starch-synthesizing enzymes towards the synthesis of starch in rice endosperm and other non-storage tissues. The detailed information on starch biosynthesis is out of purview of this article and, hence, only a brief role of enzyme isoforms is mentioned here along with efforts updates for identification of linked markers for these enzymes.

4.1. Adenosine diphosphate glucose pyrophosphorylase (AGPase)

ADP-Glc pyrophosphorylase (AGPase) consists of two large (AGP-L) and two small (AGP-S) catalytic subunits controlled by a minimum of six genes (Lee et al., 2007) (Fig. 2). Its major activity (65-95%) is performed by its cystolic isoforms which facilitates import of ADPglc into amyloplasts in the developing endosperm of rice (Lee et al., 2007; Tetlow et al., 2004; Utsumi et al., 2011). In amyloplasts, ADPglc acts as soluble precursor and substrate for the starch synthesis. Study of rice *AGP* gene family in detail revealed involvement of a total of six genes responsible for AGPase enzyme which includes two genes encoding small subunits (*OsAGPS-1* and *OsAGPS-2* [a and b]) and four genes encoding large subunit (*OsAPL-1*, *OsAPL-2*, *OsAPL-3* and *OsAPL-4*). The multiple genes showed strong tissue specificity in their expression such as the transcript of *OsAGPS-2* was found leaf specific; *OsAPL-1, OsAPL-3* and *OsAPL-4* plastid specific; and the remaining three isoforms (*OsAGPS-1, OsAGPS-2b* and *OsAPL-2*) were found to be seed specific (Lee et al., 2007; Ohdan et al., 2005) (Table 4). Analysis of *OsAGPS-2* and *OsAPL-2* mutants revealed that a lesion of one of the two cytosolic isoforms causes a shrunken endosperm due to remarkable reduction in the starch synthesis in rice endosperm. Although as per current understanding, this enzyme is responsible for transport of ADPglc into amyloplasts but still detailed study is required to enhance our current understanding on its production, activity and functionality.

4.2. Starch synthases (SS)

Ten isoforms of SS have been distinguished in rice which can be grouped into five classes i.e. SS-I, SS-II (a,b,c), SS-III (a,b), SS-IV (a,b) and granule bound SS (GBSS) (I,II) (Hirose and Terao, 2004; Nakamura, 2002; Tetlow et al., 2004; Zhang et al., 2011). These enzymes together perform a chain of reactions and catalyze the transfer of the glucosyl moiety of the soluble precursor ADPglc to the reducing end of a pre-existing ά (1–4) linked glucan primer to synthesize the insoluble glucan polymers amylose and amylopectin (Tetlow et al., 2004).

4.2.1. Starch synthase-I (SS-I)

It plays an important role in the starch biosynthesis, has no isoforms and is involved in synthesis of short chains (≤10 DP). Abundance of short chains (SC) and shortage of long chains (LC) decrease the gelatinization temperature. Soluble starch synthases (SS-I, II, III, IV) have higher affinity for the ADPglc than the GBSS and is mainly responsible for amylopectin elongation. The N-terminal extension of SSI is important for its proper binding with the starch granules (Imparl-Radosevich et al., 2003). Amylopectin chains are synthesized by the coordinated actions of SSI, SSIIa and SSIIIa isoforms, and the activity of SSI is reported to be higher than that of the SSIIa and SSIIIa enzymes (Fujita et al., 2006).

4.2.2. Starch synthase-II (SS-II)

Three isoforms (SS-IIa, IIb, IIc) for SS-II enzyme have been identified in rice. The *SS-IIb* gene is mostly expressed in leaves while substantial expression of *SS-IIa* and *SS-IIc* genes has been observed in the endosperm (Hirose and Terao, 2004; Nakamura et al., 2005; Ohdan et al., 2005; Zhang et al., 2011). The interesting and important fact is that the *SS-IIa* gene, the *alk (t)* gene (controlling alkali digestion), the *gel (t)* gene (controlling difference in gelatinization) and the *acl(t)* gene (controlling variation in the amylopectin chain length distribution i.e. ratio of short (≤11)/medium size (12–24) amylopectin chains) are mapped to the same locus on chromosome 6 in rice. The *SS-IIa* gene plays a specific role in the synthesis of the medium size glucon chains (12–24 DP) by elongating short chains (≤10 DP) (Table 4). Although *SS-IIa* gene is a minor contributor to the total SS enzyme activity in the endosperm as compared to *SS-I* and *SS-III* genes, loss/down regulation of *SS-IIa* gene has the major impact on quantity and composition of starch in rice endosperm (Yu et al., 2011).

4.2.3. Starch synthase-III (SS-III)

SS-III enzyme possesses two isoforms, i.e., SS-IIIa and SS-IIIb, in rice and is specifically expressed in the developing rice endosperm and leaf, respectively (Fujita et al., 2007; Hirose and Terao, 2004; Ohdan et al., 2005; Zhang et al., 2011). The deficiency in the SS-IIIa, the second major SS enzyme in the developing rice endosperm, affected the structure of amylopectin, amylose content and the physicochemical properties of the starch granules in two ways: directly by the SSIIIa enzyme deficiency itself, resulting in the chain length increase (DP 10–15 and DP 20–25), and indirectly by the enhancement of both the *SS-I* and *GBSS-I* gene transcripts (Fujita et al., 2007) (Table 4). It is also been suggested that the GT of the starch could be regulated by the ratio of ≤12–16 DP/>12–16 DP in the A chains and the exterior part of B chains of amylopectin (Fujita et al., 2006).

The decrease in short chains and increase in medium chains have been observed in the rice *SS-IIIa* mutants (Fujita et al., 2007).

4.2.4. Starch synthase-IV (SS-IV)

Two isoforms, i.e., SS-IVa and SS-IVb, have been identified for SS-IV enzyme. So far no mutant has been isolated with lesions in gene controlling SS-IV enzyme and, hence, no role could be assigned for this class of SS in the process of starch biosynthesis.

4.2.5. Granule bound starch synthase (GBSS)

GBSS enzyme comprises of two isoforms (GBSS-I, GBSS-II). The GBSS-I is encoded by *waxy* locus and is involved in the synthesis of long amylopectin (CL 85–180) chains (Cai et al., 1998; Denyer et al., 1996; Fu and Xue, 2010; Takeda et al., 1987; Wang et al., 1995) in higher proportion, resulting in the absence of very long chains in the waxy rices. The expression of GBSS-I enzyme appears to be confined mostly to the storage tissues, while GBSS-II enzyme is encoded by another separate gene and is responsible for the amylose synthesis in the leaves (Cai et al., 1998; Fu and Xue, 2010; Fujita and Taira, 1998; Vrinten and Nakamura, 2002). The huge variation in rice germplasm for AC is determined by the activity level of the GBSS-I, which in turn depends on the 5 alleles (Wx^a, Wx^b, Wx^{in}, Wx^{op} and *wx*) (Sano, 1984; Mikami et al., 2008; Chen et al., 2008). A microsatellite (SSR with 8–20 CT repeats) in the un-translated region of exon 1 of the *GBSS-I* gene discriminates between different types of amylose (Ayres et al., 1997). A relationship between AC and number of CT repeats was established through several studies (Ayres et al., 1997; Bao et al., 2002; Bergman et al., 2000; Bligh et al., 1995; Fitzgerald, 2004; Olsen and Purugganan, 2002) and been concluded as follow: (a) low amylose temperate *Japonica* rices possess Wx^a allele (18–19 CT), (b) medium amylose tropical *japonica* possess Wx^{in} allele (11–10 CT repeats), and (c) high amylose *Indica* rices carry Wx^b allele (14–20 CT repeats).

4.3. Starch branching enzymes (SBE)

After elongation of the glucal chains by the SS enzyme, the another enzyme, namely, SBE with two isoforms, i.e. SBE-I and SBE-II, generates ά-(1–6) linkages by cleaving internal ά-(1–4) bonds and transferring the released reducing ends to C6 hydroxyls to form the branched structure of the amylopectin molecule. SBE-II proteins transfer shorter chains and show a higher affinity towards amylopectin as compared to the SBE-I, which shows higher rates of branching with amylose (Guan and Preiss, 1993; Nakamura et al., 2010; Takeda et al., 1993; Tanaka et al., 2004; Tetlow, 2006; Tetlow et al., 2004) (Table 4). The termini (N- and C-) of these enzymes play important roles in determining the substrate preference, catalytic capacity and chain length transfer (Kuriki et al., 1997). In the monocots, the *SBE-II* gene has two closely related but distinct gene products i.e., SBE-IIa and SBE-IIb enzymes (Rahman et al., 2001). Down regulation or elimination of *SBE-I* gene activity showed minimal effects on the starch synthesis (Blauth et al., 2002; Flipse et al., 1996; Satoh et al., 2003b), while *SBE-IIa* gene showed clear/major role in the leaf starch synthesis and no effect on storage starch of endosperm (Blauth et al., 2001). The SBE-IIb enzyme has a distinct role in the transfer of short chains, which are then most probably extended by SS to form A and B1 chains of the rice amylopectin cluster structure (Nishi et al., 2001). Study on *SBE-I* mutants of rice have shown decreased level of the amylopectin chains with DP 12–20 and DP≥37, and increased level of amylopectin chains with DP 24–34 (Satoh et al., 2003a, 2003b). Hence, the role of the SBE-I enzyme is clear and important in the synthesis of B1, B2 and B3 amylopectin chains (Nakamura, 2002; Satoh et al., 2003a, 2003b). One more enzyme isoform (SBEIII) has also been reported recently (Chen et al., 2004), which plays an important role in the synthesis of 1–6 branching linkage.

4.4. Starch debranching enzymes (DBE)

The DBE catalyses the hydrolysis (debranch) of ά-(1–6) bonds in amylopectin and removal of the improper branch. Two types of DBEs have been distinguished based on their substrate specificity, namely, isoamylase and pullulanase. Isoamylase debranches glycogen, phytoglycogen and amylopectin, but does not attack pullulan, while pullulanase attacks both pullulan and amylopectin, but does not attack glycogen and phytoglycogen (Nakamura, 1996; Utsumi et al., 2011) (Table 4). Although specific roles of isoamylase and pullulanase are not yet clear, the *sugary-I* and *isoamylase-I* mutants of the rice endosperms provided evidence for involvement of both the DBEs in rice endosperm amylopectin biosynthesis (Fujita et al., 2003; Kubo et al., 1999; Nakamura, 1996; Wong et al., 2003).

5. Challenges and future prospects

Most of the economically important traits in crop plants have been genetically manipulated to improve cultivars with extreme level of phenotype such as high resistance, high protein and oil content. But in the case of improving rice grain quality, majority of the consumers in the world prefer the rice with intermediate AC and GT because of the good cooking and eating qualities. Considering the array of grain quality features either directly or indirectly affected by complex network of SS enzymes, the improvement is more cumbersome and difficult using conventional breeding approaches alone. Since the very beginning, plant breeders working on the improvement of the rice grain quality have always targeted AC and GT as a measure to assess the physicochemical/starch properties traits in the rice for selecting improved lines with desirable range of AC and GT. On the other hand, biochemists improved our understanding on roles of the starch-synthesizing enzymes in the synthesis of amylose and amylopectin, which has helped in understanding the starch biosynthesis in rice endosperm in a better way. In addition, genomic regions responsible for the cooking quality traits were identified through forward genetics approach by mapping the QTLs and genes. Using advanced genomic approaches, recent studies reported identification of starch-synthesizing genes and their isoforms by reverse genetics approach and attempted to relate their effects on rice grain quality traits. Majority of the reports clearly identified chromosome 6 to be rich in the genes related to starch properties (*GBSS-I, SSIIa* and *SBE-1*) along with other genomic regions scattered in rice genome and, hence, a holistic approach is required by pooling the information generated through forward genetics (QTLs/genes for AC and GT) and reverse genetics (genes responsible for different starch-synthesizing enzymes) approach to utilize the information for improving these traits. The genomic regions responsible for the starch properties traits (AC, GT) and starch-synthesizing enzymes (SS, SBEs, DBEs) need to be compared and perfect molecular markers should be developed, which can explain the variation for starch-synthesizing enzymes and starch properties traits together. Since the full genome sequence information is available in the public domain for an *Indica* genotype (BG-1) and a *Japonica* genotype (Nipponbare) along with large number of EST database, these databases should be used judiciously to develop tightly linked markers. Although it looks difficult to develop perfect markers for each isoforms of all the enzymes involved in starch synthesis in rice endosperm controlling desirable range of AC and GT, if done so, it will definitely help the breeders to develop rice variety with desirable cooking and eating quality with more precision using the maker-assisted selection.

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