Grain Quality

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1 Introduction

Rice is a major human food crop that feeds over 50 % of the world's population. During the past decades applications of the "green revolution" and hybrid rice technologies have rapidly improved rice productivity. However, the previous breeding goal in last century was over shed light on yield; accordingly, the rice quality was not paid more attention to and the grain quality was not increased as fast as yield. The rice qualities include the appearance, milling quality, nutritional quality, and eating and cooking quality (ECQ) [1]; appearance and ECQs have received more attention than other qualities in rice from consumers. Given that the rice endosperm is the major eating part and starch is the predominant component in dehulled grains (composed of 76.7–78.4 % in polished rice), the rice ECQs are thus thought to be mainly influenced by starch properties [2]. The improvement in rice grain quality has been increasingly demanded by consumers and has become a priority for rice breeders and geneticists [3–5]. Studies on the elucidation of the molecular mechanisms underlying appearance and ECQs of rice have made significant advances recently. This chapter will review current progress in understanding the genetics and molecular biology of rice grain quality, focusing on ECQs and appearance of rice grains.

2 ECQs of Rice Grains

2.1 Properties Affecting Rice Grain ECQs

Three physicochemical properties of starch have been considered as determinants of rice grain ECQs: amylose content (AC), gel consistency (GC), and gelatinization temperature (GT) [6, 7].

Amylase content (AC), also called AAC (apparent amylose content) because of the measurement method used to assay amylose content by iodine staining, can detect both amylose and long chain amylopectin [8]. Based on AC values, rice varieties can be divided into two classes: glutinous rice (extremely low AC, 1–2 %) and non-glutinous rice (AC >2 %). Non-glutinous rice can be further classified into four types: very low AC (2–10 %), low AC (10–20 %), medium AC (20–25 %), and high

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AC (>25 %). In general, *japonica* rice contains low to very low AC values and tends to be sticky, moist, and tender when cooked. Generally, *japonica* rice is highly preferred in northern-east Asia, including China, Japan, and Korea. In contrast, *indica* rice contains high AC and cooks soft and fluffy in texture and is favored in southern and southeastern Asian regions [9].

Gel consistency (GC), as a good index of cold paste-viscosity of cooked rice, refers to the gel running distance of digested grains by KOH in a flattened tube [10]. Based on gel length, rice varieties can be divided into three classes: hard (<40 mm), medium (40–60 mm), and soft (>60 mm). Cooked rice with hard GC tends to be dry and fluffy after cooling, whereas soft GC rice remains moist and continues to remain soft after cooling. In general, soft GC rice is more desirable by consumers [11].

Gelatinization temperature (GT) is the temperature at which starch granules start to lose crystallinity and birefringence by irreversible expansion that alters the starch surface from polarized to a soluble state [12]. To measure rice grain GT, several approaches have been developed, such as differential scanning calorimetry (DSC), rapid visco analyser (RVA), and alkali spreading value (ASV). Based on the ability of seed resistant to alkali digestion, varieties can be classified into three categories: high GT (>74 °C), intermediate GT (70–74 °C), and low GT (<70 °C) [12]. Given that resistance of seeds to alkali digestion is antagonistic with GT, high GT varieties are substantially hard to be digested when eaten, and vice versa.

In general, rice varieties with fine ECQs can be characterized as having medium AC (15–17 %), soft GC (>60 mm), and low GT (<70 °C) [13].

2.2 Genetic Molecular Studies of AC

2.2.1 Inheritance of AC

Studies on domestic varieties have shown that long grain types (*indica* rice) can be characterized by relatively high amylose content (24–28 %), whereas typical short and medium grain varieties (*japonica* rice) have relatively low amylose content (15–20 %) [14], which has accelerated the application of AC as a selection criterion in rice breeding programs [14]. At present, three inheritance models of AC have been proposed. According to the first model, high AC is controlled by a single dominant major gene, along with some minor genes and/or modifiers [15, 16]. The second model supports the existence of two dominant or complementary genes that control the AC trait [17]. The third model hypothesizes that AC is a quantitative trait controlled by multiple genes [18]. In general, the first model is more popularly accepted by geneticists and breeders [15, 16, 19–22] and is supported by the transgressive segregation in F_2 populations derived from low AC and intermediate AC parents [9, 16].

2.2.2 QTLs for AC

With the development of high-density marker linkage maps in rice, a series of studies for QTL analysis of rice grain quality have been conducted. Table 16.1 summarizes QTL that have been identified in rice for AC over the past decades. A doubled-haploid (DH) population, derived from the anther culture of an *indica*/*japonica* hybrid, was first utilized to identify QTL for AC [1] which identified two QTL on chromosomes 5 and 6. The major QTL on chromosome 6 explained 91.1 % of the variance and was closely linked with the previously identified *Wx* gene, which had been shown to control AC in both maize and rice [23, 24]. Thereafter, several groups have detected the *Wx* locus [6, 25–31] as the major AC QTL, as well as other minor effecter loci in diverse populations [1, 26, 28, 32–34]. Besides the popular DH population [6, 29–31], other populations, like F_2, BC_2, BC_3, and CSSL, have also been developed and applied for positioning of AC loci [25, 27, 33, 34]. In addition, two pairs of epistatic QTL involving QTL-by-environment interactions (QEs) of AC have been detected as well [6]. These data indicate that the regulation of AC is complex and associated with a number of heritable factors and environmental conditions.

2.2.3 Genes Regulating Amylase Content

Since the identification of two classical maize mutants defective in amylase in kernels [23] and endosperm in the last century [35], mutants with (Beijing) a similar waxy phenotype have been

Table 16.1 Identified QTL for amylose content in rice

Cross	Population type	QTLs	Chr.	Marker interval	LOD value	Additive effect	Variance explained (R2)	Reference
ZYQ8/JX17	DH	qAC-5	5	RG573-C624	2.67	−3.32	11.8	[1]
		qAC-6	6	Wx	28.39	−8.52	91.1	
Zhenshan97/Minghui63	F2&RIL	Wx	6	RM170-RM190	69	2596.2^{MS}	N.A	[25]
IR64/Azucena	DH	AAC	7	RG375/RG477	2.61	0.76	6	[32]
IR64 x Oryza rufipogon	BC2F2	AC	6	RM170	14.63	−0.88	21.9	[27]
V20A/IRGC 103544	BC3(TC)F1	qAC-6	6	RG653	4.2	0.06	8	[33]
		qAC-12	12	RG574	6.4	0.07	8	
Caiapo/IRGC103544	BC3F1/DH	qAC-3	3	RM7-RM251	3.7	−2.73	21.5	[28]
		qAC-6	6	RM190-RM253	19.3	−2.60	73.7	
		qAC-8	8	RM230-RM264	3.1	−1.85	10.9	
Asomonori/IR24	CSSL	qAC-8	8	G1149-R727	3.7	1	17.1	[34]
		qAC-9a	9	XNpb36-XNpb103	2.5	1	13.4	
		qAC-9b	9	C609-C506	2.4	1.1	19.2	
		qAC-12	12	XNpb189-2-XNpb24-2	2.5	1.4	8	
Wuyunjing2/Zhenshan97B	DH	qAC-6	6	RM190-RM510	35.5	−9.4	61.8	[29]
Nanjing11 × Balilla	DH	qAC-6	6	S10372-Wx	31	4.8	72.8	[31]
PSB Rc10/Nip	DH	qAC6a	6	RM469-RM170	38	5.88	65	[30]
		qAC6b	6	RM170-RM190	62.33	6.37	74	
		qAC6c	6	RM197-RM225	20.91	4.77	63	
Zhenshan97/H94	DH	qAC6A	6	RM190-RM587	65.8	5.83	54.87	[6]
			6	Cgene-MRG5119	6.2	0.83	1.1	
			11	RM209-RM229	10.8	−1.07	1.85	
			12	RM270-RM235	7.9	−0.73	0.85	
KDML105/CT9993	RIL	qAC3	3	RM81-C155	N.A	1.86	11.28	[26]
		Qac4	4	G177A-GA2-7	N.A	0.63	15.99	
		qAC6	6	Waxy-RM204	N.A	4.48	58.69	
		qAC7	7	OSR22-RM10	N.A	0.96	9.18	

Note: MS = MS effect

subsequently identified in rice, barley, wheat, potato, sorghum, and amaranths [24, 36–41]. The maize *Wx* gene was cloned in 1983 [42] and used subsequently as a probe to identify the homologous gene in rice [43], resulting in the identification of a 2.4-kb transcript that has been fully characterized [22, 44]. Surprisingly, besides the normal 2.4-kb transcript, an extra aberrant 4.0-kb transcript has also been found in glutinous rice from cultivar PI291667. Given that the translation of the maize and barley *Wx* genes begins in exon 2, raising the possibility that the extra-long *Wx* sequence may be attributed to the retention of the first intron [41, 45]. Sequencing the aberrant fragment of the *Wx* transcript from rice cultivar Hanfeng revealed that the entire intron 1 was indeed present in the aberrant long cDNA, and that the 3′ end of *Wx* cDNA included proper termination features of Poly(A) and an AATAAT sequence. In addition, it shares exactly the same sequence as its counterpart in non-glutinous rice [24, 44]. Furthermore, Northern-blot and Western-blot analyses of multiple varieties showed that low AC cultivars accumulate substantial amounts of un-spliced long *Wx* transcripts, including intron 1, and that high AC cultivars are depleted of the un-spliced transcript [24]. All of these data support the 5′ end retention hypothesis. Actually, previous data have shown that two Wx proteins, Wx^a and Wx^b, were present in rice. Wx^a is characteristic of *indica* rice with high AC and Wx^b is mainly found in *japonica* rice with intermediate AC [37]. In contrast to the Wx^a-type rice, a T to G change in the 5′ splice site of intron 1 was detected in Wx^b and a significant reduction of Wx^a transcript amount was also observed [46]. These data further prove that the role of the G to T change in the first intron of the *Wx* gene affects transcription levels and final amylose biosynthesis. In the rice *du1* mutant, Wx^b transcription [47] and protein accumulation were reduced significantly [48]. Therefore, *Du1* may participate in both *Wx* transcriptional and translational regulation. A recent study revealed that *Du1* encodes a Prp1 protein, a component of spliceosome. The defect of Prp1 in the *du1* mutant leads to a specific decrease of the splicing efficiency of *Wx* rather than other starch biosynthesis-related genes (SSRGs) [49]. Furthermore, the AC level of a *du1*/*wx* double mutant is almost the same as that of *Du1*/*wx* mutant, which is much lower than that of the *du1*/wx^b mutant. In mammals, the U5-102kD Prp1 protein interacts with U4/U6 snRNPs and bridges the two particles through its TPR elements [50]. However, the molecular basis of why a *Du1* mutant specifically affects the splicing of the Wx^b pre-mRNA, and how Du1 recognizes the first intron of the *Wx* transcript remains to be elucidated [49]. In addition, the MYC (for v-myc avian myelocytomatosis viral oncogene homolog) protein has been shown to interact with EREBP (for ethylene responsive element binding protein) and bind to the *Wx* gene promoter, which results in enhanced transcription of the *Wx* gene [51, 52]. All these results show that transcriptional and posttranscriptional regulation of the *Wx* gene is crucial for the expression of *Wx* and amylose biosynthesis.

2.3 Genetic Molecular Studies of Gel Consistency (GC)

2.3.1 Inheritance of GC

Gel consistency (GC) is a good index of cooked rice texture for cold paste-viscosity, especially among rice varieties with high amylose content and varieties with hard, medium, and soft GC levels have been selected for by rice breeders with their breeding goals. To explore the genetic inheritance of the three types of GC levels, bulked F_2 and F_3 seeds were analyzed and the hard GC was found to be controlled by a single dominant gene [19]. Subsequent investigations using a single grain analysis resulted in a similar conclusion [11, 53]. The inheritance of GC was further explored by utilizing various populations, such as F_2, B_1F_1, and B_2F_1, derived from parents with hard and soft, hard and medium, and medium and soft GC properties as well, and a major gene with multiple alleles was identified [54]. These studies all suggested that the hard GC is controlled by a single locus/gene. However, analyses using a 6X6 diallel set excluding reciprocals and involving contrasting parents revealed the predominance

of additive gene action in the regulation of GC trait expression [55]. A similar result was also observed by Yi and Chen [56], implying that the GC is unlikely controlled by a major gene.

2.3.2 QTL for GC

Like AC, a number of studies have been conducted to understand the genetic basis of GC [25–29, 31–34, 57, 58]. Because of the negative correlation between AC and GC, it was suggested that GC is controlled by *Wx*, or another gene closely linked to the *Wx* locus [5, 25]. To identify QTL responsible for GC, an RIL population was developed and a single QTL with a large MS effect was detected on chromosome 6, which corresponded well with the *Wx* gene [25]. Furthermore, using two additional RIL populations, major QTL for GC on chromosome 6 had been detected as well, accounting for 57 % and 53 % of the phenotypic variation, respectively [26, 57]. The *Wx* locus responsible for GC was also identified with different DH populations [5, 29, 31]. All of these results indicate that the *Wx* locus is the major candidate gene controlling GC. Besides *Wx*, several minor effect QTL (Table 16.2), located on different chromosomes, were also identified from different populations [25–29, 31–34, 57, 58]. Therein, two loci identified using a DH population from similar GC parents were found not to overlap with known SSRG genes [32]; therefore, it should be interesting to clone these novel GC regulatory genes.

2.3.3 Genes Regulating GC

Based on genetic studies, numerous reports suggested that *Wx* was the primary determinant of GC; however, no direct molecular evidence was available until recently. The major QTL on chromosome 6, qGC-6, was represented with a DH population and this locus was finally characterized by using chromosome segment substitution lines [5]. *qGC-6* encodes a granule-bound starch synthase (Wx), which has been well-documented for its role in AC. A comprehensive comparison revealed several polymorphic sites, including a previously known G/T transition between CJ06/TN1 parents. Although the complementation experiment had confirmed the role of the *Wx* gene in GC, which or how many SNP or InDel sites in the *Wx* gene are pivotal for GC and starch biosynthesis remains to be determined. Besides *Wx*, other starch-related genes, like *ALK* and *SBE3*, have been shown to play a role in regulating GC as well. When a functional *ALK* gene from low GC rice (Shuangkezao, *indica* type) was transferred into the GC intermediate Nipponbare (*japonica* type), transformants showed a decreased GC value [59]. However, the opposite phenotype was also observed in rice plants over-expressing the *ALK* gene, in which the GC value increased significantly [7]. Therefore, it will be interesting to elucidate the molecular mechanism of *ALK* in regulating GC in future. In addition, transformation of a functional *SBE3* gene into *japonica* cv. WYJ7 decreased the GC value significantly [7], indicating that *SBE* may function as a minor gene contributing to GC.

2.4 Molecular Genetic Studies of Gelatinization Temperature

2.4.1 Inheritance of GT

The inheritance of GT has been studied extensively, but its mode has not been found to be consistent, not only in the number of genes responsible for GT but also in the nature of dominance-recessive relationships [60, 61]. Puri et al. reported the segregation patterns of GT in five reciprocal cross populations derived from three different GT (high, medium, and low) parents, but and could not identify consistent mode of inheritance, suggesting the lack of a major gene in controlling GT [61]. A similar conclusion was also drawn from studies on crosses between the cultivar 9192 and the mutant *mahsuri* [60] and an *indica* cytoplasmic male sterile (CMS) line and its restorer lines, respectively [62]. However, a bimodal frequency distribution was detected in an F_2 population between SD7 and 72-3764, indicating that a major locus is governing GT [17]. Moreover, a few additive genes with major effects, along with modifier genes, were proposed as well [17]. Lastly, it should be pointed out that GT inheritance has been shown to be affected by

Table 16.2 Identified QTL for gel consistency in rice

Cross	Population type	QTLs	Chr.	Marker interval	LOD value	Additive effect	Variance explained (R2)%	Reference
Zhenshan97/Minghui63	F2:3 & RIL	qGC-6	6	c952-C1496	N.A	10722.7$^{(MS)}$	N.A	[25]
KDML105/CT9993	RIL	qGC6-1	6	Waxy-RM225	N.A	16.98	53.1	[26]
		qGC6-2	6	RG64-R2171	N.A	5.18	10.5	
		qGC7-1	7	RG375/RG477	N.A	8.20	12.77	
IR64/Oryza rufipogon	BC2F2	qGC	6	RM50	14.63	3.57	6.6	[27]
IR64/Azucena	DH	qGC-1	1	RG331/RG810	2.71	–3.7	9	[32]
		qGC-7	7	RG477/PGMSO.7	3.54	–4.65	13	
Asomonori/IR24	CSSL	qGC-4	4	C445-Ky4	4.9	1	–3.8	[34]
		qGC-6	6	XNpb209-C688	2.3	1	–3.3	
		qGC-11	11	XNpb257-C1350	8.3	1.1	–9.3	
Wuyunjing2/zhenshan97B	DH	qGC-1	1	RM294B-RM306	2.3	7.8	5.7	[29]
		qGC-2	2	RM190-RM510	2.5	7.9	6.3	
		qGC-6	6		33.7	22.4	59.7	
Zhenshan97/H94	DH	GC6a	6	RM170-RM589	7.3	–6.87	10.88	[6]
		GC6b	6	RM190-RM587	29.6	–11.87	32.52	
		GC6c	6	C gene-MRG5119	5.9	–2.08	1	
		Epistatic	1	RM543-RM302	N.A	2.5	1.45	
Nanjing11/Balilla	DH	qGC6-1	6	Wx-R1952	16.3	–12.9	47	[31]
		qGC6-2	6	RM508-RM435	6.2	–8.4	19.7	
PSB Rc10/Nip	DH	qGC2	2	RM71-RM2634	3.2	–4.58	4	[30]
		qGC6a	6	RM469-RM170	14.48	–11.44	23	
		qGC6b	6	RM170-RM190	23.81	–14.15	35	
		qGC6c	6	RM197-RM225	10.61	–11.32	23	
		qGC8	8	RM350-RM342A	3.59	–4.8	4	
Zhenshan97/Minghui63	RIL	qGC-1-1	1	C904-R2632	N.A	5.02	N.A	[57]
		qGC-6-2	6	C952-Waxy	N.A	17.2	53	
TN1/CJ06	DH	qGC-2	2	RM3732-RM492	2.96	–16.76	17.8	[5]
		qGC-3	3	RM514-RM85	3.05	–18.40	14.6	
		qGC-6	6	RM540-RM587	12.17	–32.30	52.4	

Note: MS = MS effect

environment, whereby high air temperature after flowering raises GT and lower temperatures have the opposite effect [60, 63].

2.4.2 QTLs for GT

As mentioned above, the inheritance model of GT had been well explored using crosses among diverse cultivars. To identify the genes responsible for GT, a number of QTL mapping populations (e.g., F_2, RILs, BILs, DH, and CSSLs) have been developed and are listed in Table 16.3. Among them, DH populations have been widely employed [1, 6, 29, 30, 32] and resulted in the identification of important two QTL [1]. One QTL was found to be a major contributor to GT and was delimited to the CT506-C235 interval on chromosome 6. This QTL was recognized by several labs using diverse population types [6, 26, 28, 29, 58]. The CT506-C235 region contains a known locus, *ALK*, which is responsible for alkali digestion, indicating the pivotal role of *ALK* for GT. Besides *ALK*, the *Wx* gene, with both major and minor effects, has been identified as well [6, 26, 28, 29, 32, 34].

2.4.3 Cloning ALK—A Gene That Regulates GT

To clone ALK, a locus that has been shown to regulate GT, segregating F_2 populations were utilized to map and fine map *ALK* to a 9-kb region on chromosome 6 between the genetic markers R2147 and C1478 [12, 64]. BlastX analysis revealed a partial ORF that encoded a soluble starch synthase IIa (SSIIa or ALK) within the 9-kb region and three amino acid substitutions in the ALK genes of the parental lines C Bao (low GT) and Shuangkezao (high GT) were detected [12]. Considering the previous data that ALK is functional in elongation of medium chain-length amylopectin [65], it is therefore interesting to understand the role of different amino acid substitutions in ALK in regulating chain-length elongation. To do that, an elaborate set of expression shuffle constructs with diverse amino acid residue substitutions in the wild-type cv IR36 ALK sequence were established, and the purified proteins were utilized to test their SSIIa activity in vitro [66]. Results showed that the replacement of Val-737 with Met-737 abolished SSIIa activity in chain-length elongation from the degree of polymerization (DP) <12 to DP 13–25, indicating a critical role of the Val-737 site for ALK function. In contrast, substitution of the site Phe-781 only partially affected ALK activity, and double mutations of residues Gly-604 and Phe-781 were shown to enhance the deficiency of ALK activity. These observations suggest that the Phe-781 is an important secondary site and that Gly-604 may interact positively with Phe-781 to determine ALK activity [66].

2.5 Complex Network Regulating Starch Biosynthesis

As rice grain ECQs are triploid endosperm traits, their inheritance patterns are very complicated because the genetic expression of an endosperm trait in cereal seeds is conditioned not only by the triploid endosperm genotype, but also by the diploid maternal genotype, and additional cytoplasmic components [1, 67, 68]. Several studies concerning the inheritance of rice grain ECQs have been conducted over several decades, but the data are not always consistent. The generally accepted model is that nuclear gene expression is the predominant mechanism affecting rice grain quality, though a few studies have suggested that the chloroplast genome may play a role as well [56, 69].

Based on current knowledge, starch structure is determined by four classes of enzymes: ADP glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE), and debranching enzyme (DBE) [70, 71]. As previously described, the two SS enzymes, Wx and ALK, have been well documented to affect AC and GT. Accumulating evidences have shown that Wx is involved in amylose biosynthesis, especially in the formation of extra-long chain fractions [72, 73]. In contrast, ALK has been shown to determine the elongation of short- to medium-length starch chains (DP 13–25) [12, 59, 66]. In addition to Wx and ALK, other SSRGs, such as OsSS1 [74], OsSSIIIa [75], isoamylase1 [71], branching enzyme [76, 77], and pullulanase [78], confer their unique or overlapping roles on

Table 16.3 Identified QTL for gelatinization temperature in rice

Cross	Population type	QTLs	Chr.	Marker interval	LOD value	Additive effect	Variance explained (R2)	Reference
ZYQ8/JX17	DH	qASS-6	6	CT201-RZ450	6.19	1.23	24.6	[1]
		ALK	6	CT506-C235	27.04	2.33	82.4	
Zhenshan97/Minghui63	F2&RIL	Wx	6	C952-C1496	N.A	N.A	N.A	[25]
KDML105/CT9993	RIL	qGT2	2	RG73-RM6	N.A	0.24	12.22	[26]
		qGT6a	6	C1478-RZ667	N.A	2.21	60.30	
		qGT6b	6	RM3-RM238	N.A	0.68	8.57	
IR64/Azucena	DH	qGT	6	Amy2A/RG433	2.44	−0.35	10	[32]
Caiapo/IRGC103544	BC3F1/DH	qGT6-1	6	RM190-RM253	32.5	0.87	50.1	[28]
		qGT6-2	6	RM253-RM162	10	−0.87	44	
Asomonori/IR24	CSSL	qGT-3	3	C1677-R3156	5.1	−1.9	20.5	[34]
Wuyunjing2/zhenshan97B	DH	qGT-6	6	RM276-RM121	34	3.52	80.3	[29]
Zhenshan97/H94	DH	ASV1	1	RM297-RM128	11.8	0.35	3.63	[6]
		ASV6a	6	RM190-RM587	27.1	0.7	14.22	
		ASV6b	6	RM111-RM253	31.3	−0.83	19.58	
		ASV6c	6	RM190-RM587	46.2	−1.15	38.12	
PSB Rc10/Nip	DH	qGT2	2	RM3294-RM6233	3.48	0.19	3	[30]
		qGT6a	6	RM469-RM170	10.95	0.35	9	
		qGT6b	6	RM170-RM190	19.39	0.47	16	
		qGT6c	6	RM197-RM225	3.43	0.23	4	
		qGT6d	6	RM7023-RM3330	52.52	−0.93	62	
		ASV11	11	RM202-RM484	7.9	−0.33	3.82	

Table 16.4 Characterized starch biosynthesis and regulation-related genes in rice

Classification	Gene name	Other name	Effects in fine structure of starch	Physiochemical change in mutant	References
SS	GBSSI	Wx	Amylose decreased	Decreased AC	[73]
	SSI	N.A	Decreased in DP 8-12 amylopectin; Increased in DP 6-7 and 16-19dp amylopectin	N.A	[74, 80]
	SSII-3	ALK	Amylopectin DP13-25 decreased	Decreased GT	[59, 66, 80]
	SSIIIa	N.A	DP 6-9 and DP 16-19 amylopectin decreased; DP 10-15 and DP 20-25 increased	Decreased GT	[75, 79, 80]
SBE	Sbe1	N.A	Decrease in DP >37 and DP 12-21, increased in DP <10, and slight increase in DP 24-34 amylopectin	Decreased GT	[77]
	SbeIIb	Amylose extender(ae)	Decreased in DP 8-12 amylopectin	Increased GT	[76]
DBE	Isoamylose	ISA or sugary 1	Increased in DP8-12; depleted in DP 13-23 amylopectin	Decreased GT	[71]
	Pullulanase	Pull	Increased short chains of DP ≤12	Slightly decreased GT	[83]
TF	RSR1	N.A	Increased in DP 5-8 and 18-38	Increased AC	[51]
			DP 9-17 decreased of amylopectin	Decreased GT	
	DU1	N.A	Amylose decreased	Decreased AC	[49]

the formation of fine starch structure and rice quality regulation as well (Table 16.4).

As the major SS isozyme in developing endosperm, *SS1* mutations have not been shown to have an influence on the size or shape of grains and starch granules, or on the crystallinity of endosperm starch. However, *ss1* mutants do lead to an obvious decrease in DP 8–12 chains and an increase in DP 6–7 and DP 16–19 amylopectin chains [74]. In contrast, the *ssIIIa* mutant exhibited obvious deficiency phenotypes in grain shape, and the internal amylopectin chains, DP 6–9 and DP 16–19, were shown to decrease, while DP 10–15 and DP 20–25 chains increased [75, 79]. The opposite chain-length deficiency in *ss1* and *ssIIIa* mutants strongly indicates their distinct and overlapping functions in amylopectin biosynthesis. While the double *ss1 ssIIIa* null mutant is sterile, double mutants with a leaky *ss1* and a null *ssIIIa* allele are fertile. These results further support the role of both SSI and SSIIIa in starch biosynthesis in rice endosperm and seed development [80].

SBE1 and SBEIIb are two well-characterized starch branching enzymes from rice. The mature grain of *sbe1* mutant looks like that of the wild type, not only in appearance but also in the size and weight, whereas *sbeIIb* mutant has significantly smaller kernels with a floury appearance [77]. Biochemical analysis revealed that the AC level of endosperm starch in *sbe1* mutant was similar to that of the wild type, while significant decreases in both long chains (DP >37) and short chains (DP12-21) of amylopectin were observed [77]. In contrast, the *sbeIIb* mutant was specifically reduced in short DP <17 chains, with the greatest decrease in DP 8–12 chains to alter the structure of amylopectin in the endosperm [76].

For starch DBEs, two genes, *ISA1* [71] and *PULL* [78, 81], have been well characterized. An antisense transgenic line of *isa1* contains increased levels of short chains (DP <12) and is depleted in intermediate-size chains (DP 13–23) [71, 82]. In contrast, the *pull* mutant showed an increased level of short chains of DP <13 [78, 81, 83]. The *pull*-null/mild *isa1* double mutant still retained starch in the outer layer of the endosperm tissue, while amounts of short chain amylopectin (DP ≤7) were higher than that of the *isa1* mutant. These data indicate that the function

of PULL is partially overlapping with that of ISA1 and its deficiency has less impact on the synthesis of amylopectin than that of ISA1 [83].

Even though QTL mapping and cloning of starch synthesis genes have provided useful information for rice grain quality, it has been difficult to isolate QTL/genes with minor effects in order to elucidate the complex network of starch synthesis due to limited germplasms used in single experiments. To gain a broader understanding of the starch synthesis and its regulatory network, 18 SSRG genes were selected as candidates to carry out an association study [7]. With this approach, a fine network of rice starch biosynthesis and regulation was established. As a result 10 of the 18 SSRGs have been shown to be associated with rice grain quality. Both *Wx* and *ALK* are two central determining factors affecting all three properties (AC, GC, and GT). *Wx* functions as the sole major gene for both AC and GC, but as a minor gene affecting GT, consistent with QTL mapping results, whereas ALK was found to be the sole determinant for GT, but as a minor gene affecting AC and GC. In addition, several genes were shown to be associated with minor effect on starch biosynthesis: *SSIII-2*, *AGPlar*, *PUL,* and *SS1* for AC; *AGPiso*, *ISA,* and *SBE3* for GC; and *SSIV-2*, *ISA,* and *SBE3* for GT [7]. So far, this is the first genome-wide study of how the allelic diversity of SSRGs has collectively been shown to regulate rice grain quality via the starch biosynthesis network.

3 Grain Appearance

3.1 Features Affecting Rice Grain Appearance

The quality of grain appearance is mostly determined by grain shape as specified by grain length (GL), grain width (GW), grain length/width ratio (GS), and grain chalkiness [84]. Although preferences for rice grain appearance vary by consumers, long and slender rice is generally preferred by most of consumers in north-America and Asian countries [84]. Based on the Chinese national criteria for rice quality, the grain length of grade I rice is 6.5–7.5 mm for *indica* and 5.0–5.5 mm for *japonica* rice; and the ratio of grain length/width is >3.0 for *indica* and 1.5–2.0 for *japonica* rice.

3.2 Inheritance of Grain Shape

Grain shape is determined by grain length, grain width, and/or the ratio of grain length to width. Studies on grain shape have been explored extensively [17, 85–87], not only because of its elegant appearance from a visual sense, but also because of its strong effect on yield improvement due to its positive correlation with grain weight [88]. Genetic studies from different crosses among *japonica* X *japonica* and *indica* X *japonica* have showed that there are no obvious differences in reciprocal backcross progeny and that continuous distribution patterns were observed in progeny populations, suggesting that grain length is governed by quantitative maternal nucleic genes [89–91]. Similarly, observations using F_2 populations derived from crosses between varieties with different grain width also detected a continuous grain width distribution, suggesting a polygene model for grain width [17, 86, 92–94]. However, numbers of genes responsible for grain shape appear to be variable and are probably dependent on their genetic backgrounds. For example, Liu [95] found that the grain length segregation ratio in a specific F_2 population was 3:1 suggestive of a single gene model controlling grain shape, whereas Xu et al. found a transgressive segregation which correlated with a QTL gene model that controlled grain shape in a similar F_2 population [90].

3.3 QTLs for Grain Shape

Over 20 QTL mapping studies have been conducted to understand the genetics of grain shape and hundreds of responsible loci had been detected: 119 for grain length, 90 for grain width, and 60 for grain length/width ratio [96]. Among these studies, some QTL have been shown to account for major effects (Table 16.5) [1, 84,

97–103]. Lin et al. utilized F_2 populations derived from two pairs of *indica* parents with significant differences in grain shape to detect QTLs affecting grain length, width, and thickness. Consequently, 14 QTLs were detected [99], 5 for grain length, 2 major and 2 minor genes for grain width, and 1 major and 4 minor genes for grain thickness. Huang et al. developed a DH population from IR64 and *Azucena* parents for QTL mapping. Twelve QTL affecting grain shape were localized onto 5 different chromosomes, among them 4 for grain length, 5 for grain width, and 3 for length/width ratio [104]. Using an F_2 population, Redona and Mackill [100] identified 7 QTL for GL, of which two loci on chromosomes 3 and 7 with high LOD values had already been identified by Takeda and Saito [105] and Takamure and Kinoshita [106]. It should be pointed out that the QTL on chromosome 7 for GL also affected GW and grain length/width ratios as well. Xing et al. [107] utilized an RIL population to analyze QTL for grain shape and identified a major QTL (GW5) on chromosome 5 which is responsible for all three features of grain shape indicating that these loci may function as positive regulators to increase grain weight. By using $F_{2:3}$ and RIL populations derived from crosses between Zhenshan97 and Minghui63, a major QTL for GL on chromosome 3 (GS3) and a major QTL for grain width on chromosome 5 (GW5) were identified [84]. GS3 was also detected by several groups using different mapping populations [28, 84, 104, 108–110], indicating its general role in grain length determination. In addition, several independent studies identified a number of QTL for rice grain width using diverse mapping populations [96]. Among them, WG5 and WG7, which were first described by Lin using an F_2 population derived from Tesanai 2×CB1128 [99], were found to have significant contributions to the total grain width [84, 101, 103]. Recently, by using an F_2 population derived from Zhonghua11×Baodali (a variety with larger grain size), two major QTL for GW located on chromosomes 3 and 6 were identified [97]. In addition, a major QTL on chromosome 8 (GW8) has been detected by several groups in a number of populations [111]. These QTL have laid a solid foundation for further gene cloning and understanding of the regulation of grain width.

3.4 Genes Affecting Rice Grain Shape

Because of the strong correlation between grain shape and yield, significant efforts have been made to fine map and clone genes that regulate grain shape [110–113]. To clone the major QTL for grain length, *GW3.1* or *GS3* [84, 102, 114] was first fine-mapped to a 93.8-kb interval on chromosome 3 using a BC_2F_2 population derived from a cross between *Jefferson* and *O. rufipogon*. GS3 was then cloned using an F_2 population derived from Minghui63 and Chuan7 as was proposed to be a loss-of-function mutation of a putative transmembrane protein [113]. Protein domain analysis indicated that GS3 may have four putative domains: a plant-specific organ size regulation (OSR) domain at the N terminus, a transmembrane domain, a tumor necrosis factor receptor family cysteine-rich domain, and a von Willebrand factor type C (VWFC) domain at the c-terminus. To elucidate the roles of these domains, a series of transformation assays with different protein truncations were conducted, showing that the OSR domain is essential and sufficient for GS3 to function as a negative grain size regulator [112].

A number of studies have been reported on the mapping of GW QTL (Table 16.5). The *GW2* gene was first cloned by using a BC_3F_2 population derived from a cross between WY3 and Fengaizhan [115]. *GW2* encodes a RING-type E3 ubiquitin ligase and WY3 GW2, truncated by 310 amino acids, still possesses intrinsic E3 ligase activity, suggesting that the C-terminal of GW2 is not essential for substrate degradation. Mutations of *GW2* result in increased cell numbers and acceleration of grain milk filling rate, which in turn enhances grain width, and yield [115]. Shomura et al. [101] performed a QTL analysis with an F_2 population derived from Nipponbare×Kasalath and identified and subsequently cloned *qSW5* (*GW5*), which explained 38 % of the natural variation in the F_2 population. Sequence compar-

Table 16.5 Major QTL identified for grain shape in rice

Cross	Population type	QTLs	Chr.	Marker interval	LOD value	Additive effect	Variance explained (R2)%	Reference
Tesanai/CB1128	F_2	wg5	5	RG9-RG182	6.19	−0.14	19.7	[99]
		wg7	7	RG650-RG4	11	−0.17	32.5	
Labelle/BlackGora	F_2	GL-3	3	RZ452-RZ284	9.95	−0.29	20.9	[100]
		GL-7	7	RG711-RG650	8	−0.23	17.2	
		GB-7	7	RG711-RG650	10.54	0.13	22	
		GS-3	3	RZ403-RZ452	10.19	−0.18	21.4	
		GS-7	7	RG711-RG650	12.89	−0.08	26.2	
Zhenshan97/Minghui63	$F_{2:3}$	GL3	3	RG393/C1087	41	−0.57	63.8	[84]
		GW5	5	RG360-C734a	20.6	0.18	55.2	
Zhenshan97/Minghui63	RIL	GL3	6	RG393/C1087	33.8	−0.88	57.6	
		GW5	12	RG360-C734a	16.5	0.31	44	
Jefferson/O.rufipogon	BC_2	Gw3.1	3	RZ672-RZ474	6.49	N.A	10.9	[109]
		GW3.2	3	RM130-RG1356	6.69	N.A	11.3	
Nipponbare/Kashlath	NIL	Tgw6	6	C358	4.12	N.A	18.7	[98]
Asominori/IR24	CSSL	qGL-1	1	R210-C955	5.3	−0.15	18.2	[103]
		qGL-3	3	R19-C1677	5.9	0.29	32.8	
		qGW-5	5	R3166-R569	5.4	−0.16	27	
Asominori/IR24	CSSL	qGL-3a	3	C80-C1677	27.79	−0.26	32.2	[102]
		qGL-7	7	XNpb379-XNpb268	15.97	−0.2	19.1	
		qGL-9	9	XNpb339-C796C	12.16	0.17	10.7	
Nipponbare/Kashlath	F_2	qSW-5	5	C263-R413	N.A	N.A	38.5	[101]
Baodali/Zhonghua11	F_2	GW3	3	RM282-RM6080	N.A	N.A	N.A	[97]
		GW6	6	RM6836-RM1340	N.A	N.A	N.A	

Wg weight of grain, *GL* grain length, *GB* grain breadth, *GW* grain width, *SW* seed width

ison of *qSW5* between the two parents revealed a 1,212-bp deletion in (Nipponbare) and several SNPs. Further complementation experiments and sequencing of *qSW5* from additional cultivars revealed that the 1,212-bp deletion played an important historical role in rice domestication [101]. To understand the grain width difference between Asominori and IR24, Weng et al. [116] also found the same 1,212-bp deletion in the *GW5* gene [116]. Recently, two previously identified GW QTLs, GS5 and GW8, were fine-mapped and cloned [84, 107, 117, 118]. GS5, which encodes a putative serine carboxypeptidase belonging to the peptidase S10 family and has a PF00450 consensus domain, may function as a positive regulator of grain size by affecting grain width, filling, and weight [117]. In addition, sequencing the promoters of 51 rice accessions from diverse geographic regions identified three haplotypes that appear to be associated with grain width [117].

Previous studies revealed a major QTL *GW8.1* for grain width [119], which was fine-mapped to a 306-kb region [111] on chromosome 8. However, there is no report to date on the cloning of this gene. Recently, Wang et al. [118] reported the cloning of a major gene (*GW8*) on chromosome 8, which does not appear to be allelic to *GW8.1*. GW8 encodes a Squamosa promoter-binding protein-like 16, which belongs to the SBP domain family of transcription factors and shares homology with TGA1, a domestication syndrome gene associated with the formation of naked grains in maize.

4 Perspective for Rice Quality Improvement

Development of new cultivars with improved grain quality for eating, cooking, and grain shape is critical for rice production. Although significant efforts have been made to understand the nature of grain quality, a comprehensive molecular understanding of these phenotypes remains elusive. For example, "Yangzhou fried rice," a popular food cooked with long grain rice, has better palatability and morphology than short grain rice (Fig. 16.1). Those observations lead to the conclusion that the network of starch biosynthesis and rice quality regulation is complex. In this chapter we summarized what is presently known about the inheritance and molecular basis of grain quality characteristics and outlined the strategies for the development of high-quality rice in the future.

QTL mapping has been extensively and successfully applied to clone major genes that affect grain quality, especially for grain shape and ECQ. Unfortunately this approach has achieved limited success toward the cloning of minor grain quality genes. Two recently developed techniques, i.e., co-expression and association mapping, have been shown to be very useful for the identification of ECQ genes [7, 51]. It is therefore expected that fine-scale regulators that control grain quality will soon be identified by employing these methods, and subsequently cloned.

Even though rice supplies about 20 % of the world's dietary energy and is a good source of thiamine, riboflavin, and niacin [120], its protein content is much lower than other cereal crops (only 6.3–7.1 % in milling rice). More importantly, multiple micronutrient factors for human health like vitamin A, B, C, and D are defective in milled rice grain. For example, vitamin A deficiency in humans exacerbates afflictions such as diarrhea, respiratory disease, and childhood diseases such as measles [121]. Therefore, an important goal in rice grain quality research and breeding programs is to improve micronutrient content in rice endosperm. Efforts to improve vitamin A content in rice endosperm have been highly successful by the use of recombinant DNA technology to introduce 3 essential β-carotene biosynthetic pathway genes (PSY, LCY, CRTL) into the rice genome [121]. In addition, rice endosperm protein content has been significantly increased by transformation of the β-phaseolin seed storage protein gene from common bean [122, 123]. These examples demonstrate that it is possible to significantly improve rice endosperm grain quality and opens the door to engineer additional micronutrient and protein enhancements in the future.

With the world's population expected to increase from 7 to 9 billion inhabitants by 2050, rice breeders have been challenged to produce

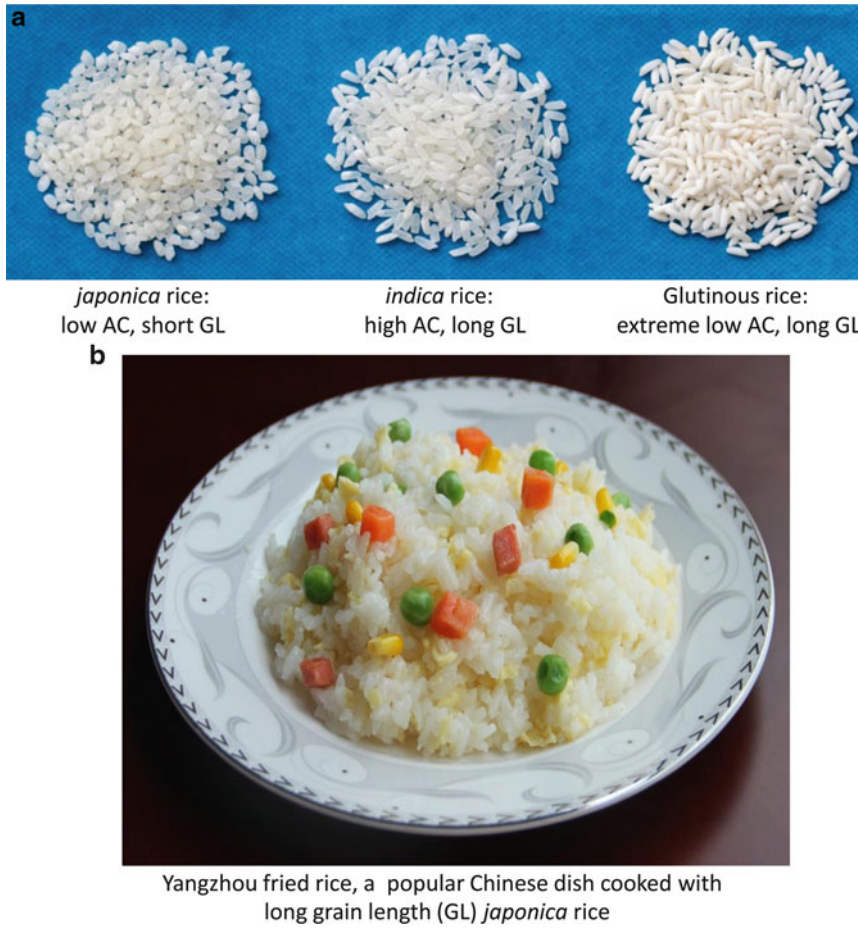

Fig. 16.1 (**a**) Three major types of rice showing their different morphologies and properties. (**b**) Yangzhou fried rice, a popular Chinese dish cooked with long grain length (GL) *japonica* rice

new cultivars that can grow with less water, fertilizer, and pesticides and have doubled yields. This challenge must also account for global warming. Preliminary data has shown that high temperatures have adverse effects on rice productivity and quality [124–128], such as the decreased brown rice rate and milling rice rate. The comparison of grain quality gene expression patterns under high and low temperatures showed that the grain quality deterioration pathway may proceed through increased sucrose synthase activity and different SSRG gene expression [127, 129]. Therefore, it is extremely important to investigate the molecular mechanisms of how high temperature signal transduction affects grain quality, which in turn will facilitate the development of new elite cultivars that have higher productivity, better quality, richer nutrients, and greater adaptation to global climate changes.

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References

1. He P, Li SG, Qian Q, Ma YQ, Li JZ (1999) Genetic analysis of rice grain quality. Theor Appl Genet 98:502–508
2. Sun MM, Abdula SE, Lee HJ et al (2011) Molecular aspect of good eating quality formation in japonica rice. PLoS One 6(4):e18385

3. Terada R, Nakajima M, Isshiki M, Okagaki RJ, Wessler SR, Shimamoto K (2000) Antisense waxy genes with highly active promoters effectively suppress waxy gene expression in transgenic rice. Plant Cell Physiol 41(7):881–888
4. Itoh K, Ozaki H, Okada K, Hori H, Takeda Y, Mitsui T (2003) Introduction of Wx transgene into rice wx mutants leads to both high- and low-amylose rice. Plant Cell Physiol 44(5):473–480
5. Su Y, Rao Y, Hu S et al (2011) Map-based cloning proves qGC-6, a major QTL for gel consistency of japonica/indica cross, responds by Waxy in rice (Oryza sativa L.). Theor Appl Genet 123(5):859–867
6. Fan CC, Yu XQ, Xing YZ, Xu CG, Luo LJ, Zhang Q (2005) The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. Theor Appl Genet 110(8):1445–1452
7. Tian Z, Qian Q, Liu Q et al (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proc Natl Acad Sci U S A 106(51):21760–21765
8. Takeda Y, Hizukuri S, Juliano BO (1987) Structures of rice amylopectins with low and high affinities for iodine. Carbohydr Res 168(1):79–88
9. Kumar I, Khush GS (1988) Inheritance of amylose content in rice (Oryza sativa L.). Euphytica 38:261–269
10. Bao J (2007) Progress in studies on inheritance and improvement of rice starch quality. Mol Plant Breed 5(6):1–20
11. Tang SX, Khush GS, Juliano BO (1991) Genetics of gel consistency in rice (Oryza sativa L.). J Genet 70(2):69–78
12. Gao Z, Zeng D, Cui X et al (2003) Map-based cloning of the ALK gene, which controls the gelatinization temperature of rice. Sci China C Life Sci 46(6):661–668
13. Gu MH, Liu QQ, Yan CJ, Tang SZ (2010) Grain quality of hybrid rice: genetic variation and molecular improvement. In: Xie F, Hardy B (eds) Accelerating hybrid rice development, Los Banos (Philippines): International Rice Research Institute, p 345–356
14. Williams V, Wu W, Tsai H, Bates H (1958) Varietial differences in amylose content of rice starch. J Agric Food Chem 6:47–48
15. Kumar I, Khush GS, Juliano BO (1987) Genetic analysis of waxy locus in rice. Theor Appl Genet 73:481–488
16. Kumar I, Khush IS (1986) Genetics of amylose content in rice. J Genet 65:1–11
17. Mckenzie KS, Rutger JN (1983) Genetic analysis of amylose content, alkali spreading score, and grain dimension in rice. Crop Sci 23:306–313
18. Bollich C, Webb B (1973) Inheritance of amylose in two hybrid populations of rice. Cereal Chem 50:631–636
19. Chang W, Li W (1981) Inheritance of amylose content and gel consistency in rice. Bot Bull Acad Sin 22:35–47
20. Huang C, Li R (1990) The genetic analysis of amylose content of rice (Oryza sativa L.). J South China Agric Univ 11(1):23–29
21. Shen Y, Min S, Xiong Z, Luo Y (1990) Genetical studies on amylose content of rice grain and modifies on the determination method. Sci Agric Sin 23(1):60–68
22. Wang ZY, Wu ZL, Xing YY et al (1990) Nucleotide sequence of rice waxy gene. Nucleic Acids Res 18(19):5898
23. Sprague GF, Brimhall B, Hoxon RMB (1943) Some effects of the waxy gene in corn on properties of endosperm starch. J Am Soc Agron 35:817–822
24. Wang ZY, Zheng FQ, Shen GZ et al (1995) The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. Plant J 7(4):613–622
25. Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang Q (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid Shanyou 63. Theor Appl Genet 99:642–648
26. Lanceras JC, Huang ZL, Naivikul O, Vanavichit A, Ruanjaichon V, Tragoonrung S (2000) Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). DNA Res 7(2):93–101
27. Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the Oryza sativa variety IR64 and the wild relative O. rufipogon. Theor Appl Genet 107(8):1433–1441
28. Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH (2004) QTL mapping of grain quality traits from the interspecific cross Oryza sativa x O. glaberrima. Theor Appl Genet 109(3):630–639
29. Tian R, Jiang G-H, Shen L-H, Wang L-Q, He Y-Q (2005) Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Mol Breed 15:117–124
30. Lapitan VC, Redona ED, Abe T, Brar DS (2009) Mapping of quantitative trait loci using a doubled-haploid population from the cross of India and japonica cultivars of rice. Crop Sci 49:1619–1628
31. He Y, Han Y, Jiang L, Xu C, Lu J, Xu M (2006) Functional analysis of starch-synthesis genes in determining rice eating and cooking qualities. Mol Breed 18:277–290
32. Bao JS, Wu YR, Hu B, Wu P, Cui HR, Shu QY (2002) QTL for rice grain quality based on a DH population derived from parents with similar apparent amylose content. Euphytica 128:317–324
33. Li J, Xiao J, Grandillo S et al (2004) QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (O. sativa L.) and African (O. glaberrima S.) rice. Genome 47(4):697–704
34. Wan XY, Wan JM, Su CC et al (2004) QTL detection for eating quality of cooked rice in a population of

chromosome segment substitution lines. Theor Appl Genet 110(1):71–79
35. Nelson OE, Rines HW (1962) The enzymatic deficiency in the waxy mutant of maize. Biochem Biophys Res Commun 9:297–300
36. Konishi Y, Nojima H, Okuno K, Asaoka M, Fuwa H (1985) Characterization of starch granules from waxy, non-waxy and hybrid seeds of Amaranths hypochrandriaces L. Agric Biol Chem 49:1965–1971
37. Sano Y (1984) Differential regulation of waxy gene expression in rice endosperm. Theor Appl Genet 68:467–473
38. Hseih JS (1988) Genetic studies on the Wx gene of sorghum. 1. Examination of the protein product of the waxy locus. Bot Bull Acad Sin 29:293–299
39. Ainsworth CC, Gale MD, Baird S (1983) The genetics of B amylase isozymes in wheat. I. Altelic variation among hexaploid varieties and intrachromosomal genelocations. Theor Appl Genet 66:39–49
40. Hovenkamp-Hermelink JHM, Jacobsen E, Ponstein AS et al (1987) Isolation of an amylose-free mutant of potato (Solanum tuberoum). Theor Appl Genet 75:217–221
41. Rohde W, Becker D, Salamini F (1988) Structural analysis of the waxy locus from Hordeum vulgare. Nucleic Acids Res 16(14B):7185–7186
42. Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the Waxy locus in maize. Cell 35(1):225–233
43. Okagaki RJ, Wessler SR (1988) Comparison of non-mutant and mutant waxy genes in rice and maize. Genetics 120(4):1137–1143
44. Okagaki RJ (1992) Nucleotide sequence of a long cDNA from the rice waxy gene. Plant Mol Biol 19(3):513–516
45. Yan L, Bhave M (2000) Sequences of the waxy loci of wheat: utility in analysis of waxy proteins and developing molecular markers. Biochem Genet 38(11–12):391–411
46. Isshiki M, Morino K, Nakajima M et al (1998) A naturally occurring functional allele of the rice waxy locus has a GT to TT mutation at the 5′ splice site of the first intron. Plant J 15(1):133–138
47. Satoh H, Omura T (1981) New endosperm mutations induced by chemical mutagen in rice, Oryza sativa L. Jpn J Breed 31:316–326
48. Sano Y, Maekawa M, Kikuchi H (1985) Temperature effects on the Wx protein level an amylose content in the endosperm of rice. J Hered 76:221–223
49. Zeng D, Yan M, Wang Y, Liu X, Qian Q, Li J (2007) Du1, encoding a novel Prp1 protein, regulates starch biosynthesis through affecting the splicing of Wxb pre-mRNAs in rice (Oryza sativa L.). Plant Mol Biol 65(4):501–509
50. Makarov EM, Makarova OV, Achsel T, Luhrmann R (2000) The human homologue of the yeast splicing factor prp6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein-protein interactions. J Mol Biol 298(4):567–575
51. Fu FF, Xue HW (2010) Coexpression analysis identifies Rice Starch Regulator1, a rice AP2/EREBP family transcription factor, as a novel rice starch biosynthesis regulator. Plant Physiol 154(2):927–938
52. Zhu Y, Cai XL, Wang ZY, Hong MM (2003) An interaction between a MYC protein and an EREBP protein is involved in transcriptional regulation of the rice Wx gene. J Biol Chem 278(48):47803–47811
53. Tang S (1993) Inheritance of gel consistency in indica rice(Oryza sativa L.). Acta Agron Sin 19(2):119–124
54. Tang S, Zhang K, Yu H (1996) Genetics of gel consistency in the crosses between indica and japonica rices. Sci Agric Sin 29(5):51–55
55. Zaman FU, Siddiq EA, Prasad AB (1985) Genetical analysis of gel consistency in rice. Indian J Genet Plant Breed 45(1):111–118
56. Yi X, Chen F (1992) Genetical effect of different cytoplasms on rice cooking, milling and nutrient qualities in indica type hybrid rice. Chin J Rice Sci 4:187–189
57. Zheng X, Wu JG, Lou XY, Xu HM, Shi CH (2008) The QTL analysis on maternal and endosperm genome and their environmental interactions for characters of cooking quality in rice (Oryza sativa L.). Theor Appl Genet 116(3):335–342
58. Victoria CL, Edilberto DR, Toshinori A, Darshan SB (2009) Mapping of quantitative trait loci using a doubled-haploid population from the cross of India and japonica cultivars of rice. Crop Sci 49:1619–1628
59. Gao Z, Zeng D, Cheng F et al (2011) ALK, the key gene for gelaterization temperature, is a modifier gene for gel consistency in rice. J Integr Plant Biol 53(9):756–765
60. Faruq G, Hadjim MO, Meisner CA (2004) Inheritance of gelatinization temperature in rice. Int J Agri Biol 6(5):810–812
61. Puri RP, Siddiq EA (1980) Inheritance of gelatinization temperature in rice. Indian J Genet Plant Breed 40(2):450–455
62. Wang C, Gao R, Tang Y (1996) Genetic analysis of gelatinization temperature of indica type hybrid rice. Acta Univ Agric Boreali-occidentalis 24(1):28–32
63. Jennings P, Coffman W, Kauffman H (1979) Rice improvement. International Rice Research Institute, Los Banos, p 186
64. Lin SY, Nagamura Y, Kurata N et al (1994) DNA markers tightly linked to genes, Ph, alk and Rc. Rice Genet Newsl 11:108
65. Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y (2002) Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor Appl Genet 104(1):1–8
66. Nakamura Y, Francisco PB Jr, Hosaka Y et al (2005) Essential amino acids of starch synthase IIa differentiate amylopectin structure and starch quality between

japonica and indica rice varieties. Plant Mol Biol 58(2):213–227
67. Pooni H, Kumar I, Khush I (1992) A comprehensive model for disomically inherited metrical traits expressed in triploid tissues. Heredity 69:166–174
68. Zhu J, Weir B (1994) Analysis of cytoplasmic and maternal effects II. Genetic models for triploid endosperm. Theor Appl Genet 89:160–166
69. Huang F, Sun Z, Hu P, Tang S (1998) Present situations and prospect for the research on rice grain quality forming. Chin J Rice Sci 12(3):172–176
70. Smith AM, Denyer K, Martin C (1997) The synthesis of the starch granule. Annu Rev Plant Physiol Plant Mol Biol 48:67–87
71. Fujita N, Kubo A, Suh DS et al (2003) Antisense inhibition of isoamylase alters the structure of amylopectin and the physicochemical properties of starch in rice endosperm. Plant Cell Physiol 44(6):607–618
72. Denyer K, Clarke B, Hylton C, Tatge H, Smith AM (1996) The elongation of amylose and amylopectin chains in isolated starch granules. Plant J 10(6):1135–1143
73. Hanashiro I, Itoh K, Kuratomi Y et al (2008) Granule-bound starch synthase I is responsible for biosynthesis of extra-long unit chains of amylopectin in rice. Plant Cell Physiol 49(6):925–933
74. Fujita N, Yoshida M, Asakura N et al (2006) Function and characterization of starch synthase I using mutants in rice. Plant Physiol 140(3):1070–1084
75. Fujita N, Yoshida M, Kondo T et al (2007) Characterization of SSIIIa-deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm. Plant Physiol 144(4):2009–2023
76. Nishi A, Nakamura Y, Tanaka N, Satoh H (2001) Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. Plant Physiol 127(2):459–472
77. Satoh H, Nishi A, Yamashita K et al (2003) Starch-branching enzyme I-deficient mutation specifically affects the structure and properties of starch in rice endosperm. Plant Physiol 133(3):1111–1121
78. Li QF, Zhang GY, Dong ZW et al (2009) Characterization of expression of the OsPUL gene encoding a pullulanase-type debranching enzyme during seed development and germination in rice. Plant Physiol Biochem 47(5):351–358
79. Ryoo N, Yu C, Park CS et al (2007) Knockout of a starch synthase gene OsSSIIIa/Flo5 causes white-core floury endosperm in rice (Oryza sativa L.). Plant Cell Rep 26(7):1083–1095
80. Fujita N, Satoh R, Hayashi A et al (2011) Starch biosynthesis in rice endosperm requires the presence of either starch synthase I or IIIa. J Exp Bot 62(14):4819–4831
81. Kubo A, Fujita N, Harada K, Matsuda T, Satoh H, Nakamura Y (1999) The starch-debranching enzymes isoamylase and pullulanase are both involved in amylopectin biosynthesis in rice endosperm. Plant Physiol 121(2):399–410
82. Wong K, Kubo A, Jane J, Harada K, Satoh H, Nakamura Y (2003) Structures and properties of amylopectin and phytoglycogen in the endosperm of sugary-1 mutants of rice. J Cereal Sci 37(2):139–149
83. Fujita N, Toyosawa Y, Utsumi Y et al (2009) Characterization of pullulanase (PUL)-deficient mutants of rice (Oryza sativa L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. J Exp Bot 60(3):1009–1023
84. Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang Q (2000) Genetic bases of appearance quality of rice grains in Shanyou 63, a elite rice hybrid. Theor Appl Genet 101:823–829
85. Qi Z, Li B, Yang W, Wu X (1983) A study on the genetic of exterior quality and fat of the rice grains. Acta Genet Sin 10(6):452–458
86. Shi C, Shen Z (1995) Inheritance and improvement of grain shape in indica rice. Chin J Rice Sci 9(1):27–32
87. Zhou Q, An H, Zhang Y, Shen F (2000) Study on heridity of morphological characters of rice grain. J Southwest Agric Univ 22(2):102–104
88. Peng X, Zou X, He H et al (2005) Analysis of genetics and correlation on grain characters in two-line hybrid rice. Hybrid Rice 20(3):60–63
89. Wu C (2002) Headways of quality heredity studding and discuss of improvement strategy in rice (Oryza sativa L.). Chin Agric Sci Bull 18(6):66–71
90. Xu C, Mo H, Zhang A, Zhu Q (1995) Genetical control of quality traits of rice grains in indica-japonica hybrid. Acta Genet Sin 22(3):192–198
91. Zhang A, Xu C, Mo H (1999) Genetic expression of several quality traits in indica-japonica hybrids. Acta Agron Sin 25(4):401–407
92. Min C, Zhao A (1983) Diallel analysis of grain weight and shape in F1 of indica rice hybridization. Sci Agric Sin 5:14–20
93. Takite T (1989) Breeding for grain shape in rice. J Agri Sci 44(6):39–42
94. Gui S, Yan K (1984) The primary study on the inheritance of giant grain in rice. Yunnan Agri Sci and Tech 1:2–5
95. Liu Y (2001) Genetic analysis of grain traits and gene mapping of grain length in rice. Master Thesis, Sichuan Agricultural University
96. Zhang Z (2008) Mapping of major QTL for grain shape and weight traits in rice (Oryza sativa L.). Master Thesis, Xiamen University
97. Guo L, Ma L, Jiang H et al (2009) Genetic analysis and fine mapping of two genes for grain shape and weight in rice. J Integr Plant Biol 51(1):45–51
98. Ishimaru K (2003) Identification of a locus increasing rice yield and physiological analysis of its function. Plant Physiol 133(3):1083–1090
99. Lin H, Min S, Xiong Z et al (1995) RFLP mapping of QTLs for grain shape traits in indica rice. Sci Agric Sin 28(4):1–7

100. Redona ED, Mackill DJ (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. Theor Appl Genet 96:957–963
101. Shomura A, Izawa T, Ebana K et al (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40(8):1023–1028
102. Wan XY, Wan JM, Jiang L et al (2006) QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. Theor Appl Genet 112(7):1258–1270
103. Wan XY, Wan JM, Weng JF et al (2005) Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theor Appl Genet 110(7):1334–1346
104. Huang N, Parco A, Mew T et al (1997) RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown plant hopper resistance in a doubled haploid rice population. Mol Breed 3:105–113
105. Takeda K, Saito K (1980) Major genes controlling grain size in Japan. Jpn J Breed 30:280–282
106. Takamure I, Kinoshita T (1991) Linkage analysis in chromosomes 3 and 6. Rice Genet Newsl 8:98–100
107. Xing Y, Tan Y, Xu C, Hua J, Sun X (2001) Mappng quantitative trait loci for grain apperance traits of rice using a recombinant inbred line population. Acta Bot Sin 43(8):800–845
108. Kubo T, Takano KN, Yoshimura A (2001) RFLP mapping of genes for long kernel and awn on chromosome 3 in Rice. Rice Genet Newsl 18:26–28
109. Thomson MJ, Tai TH, McClung AM et al (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between Oryza rufipogon and the Oryza sativa cultivar Jefferson. Theor Appl Genet 107(3):479–493
110. Li J, Thomson M, McCouch SR (2004) Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. Genetics 168(4):2187–2195
111. Xie X, Song MH, Jin F et al (2006) Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between Oryza sativa and Oryza rufipogon. Theor Appl Genet 113(5):885–894
112. Mao H, Sun S, Yao J et al (2010) Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc Natl Acad Sci U S A 107(45):19579–19584
113. Fan C, Xing Y, Mao H et al (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112(6): 1164–1171
114. Zhou LQ, Wang YP, Li SG (2006) Genetic analysis and physical mapping of Lk-4(t), a major gene controlling grain length in rice, with a BC2F2 population. Yi Chuan Xue Bao 33(1):72–79
115. Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39(5):623–630
116. Weng J, Gu S, Wan X et al (2008) Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Res 18(12):1199–1209
117. Li Y, Fan C, Xing Y et al (2011) Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nat Genet 43(12):1266–1269
118. Wang S, Wu K, Yuan Q et al (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet 44(8):950–954
119. Cho Y, Suh J, Choi I et al (2003) QTLs analysis of yield and its related traits in wild rice relative Oryza rufipogon. Treat Crop Res Korea 4:19–29
120. Wang W, Zhao Q, Yu J, Zhu D (2004) Advances in the study on the improving nutritional quality of rice. China Biotech 24(5):30–33
121. Ye X, Al-Babili S, Kloti A et al (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305
122. Zheng Z, Sumi K, Tanaka K, Murai N (1995) The bean seed storage protein [beta]-phaseolin is synthesized, processed, and accumulated in the vacuolar type-II protein bodies of transgenic rice endosperm. Plant Physiol 109(3):777–786
123. Momma K, Hashimoto W, Ozawa S et al (1999) Quality and safety evaluation of genetically engineered rice with soybean glycinin: analyses of the grain composition and digestibility of glycinin in transgenic rice. Biosci Biotechnol Biochem 63(2):314–318
124. Song J, Le M, Fu G, Wang X, Tao L (2011) The progress of molecular basis of high temeprature on rice grain quality. China Rice 15(6):8–14
125. Li H, Chen Z, Hu M et al (2011) Different effects of night versus day high temperature on rice quality and accumulation profiling of rice grain proteins during grain filling. Plant Cell Rep 30(9):1641–1659
126. Teng Z, ZHI L, Zong X, Wang S, He G (2008) Effects of high temperature on chlorophyll fluorescence, active oxygen resistance activity, and grain quality in grain-filling periods in rice plants. Acta Agron Sin 34(9):1662–1666
127. Li T, Liu Q, Ohsugi R, Yamagishi T, Sasaki H (2006) Effect of high temperature on sucrose content and sucrose-cleaving enzymes activity in rice during grain filling stage. Chin J Rice Sci 20(6):626–630
128. Peng S, Huang J, Sheehy JE et al (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci U S A 101(27): 9971–9975
129. Wei K, Zhang Q, Cheng F, Chen N, Xie L (2009) Expression profiles of rice soluble starch synthase (SSS) genes in response to high temperature stress at filling stage. Chin J Rice Sci 35(1):18–24