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Genes and QTLs for Rice Grain Quality Improvement

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1. Introduction

As a major cereal crop, rice (*Oryza sativa* L.) is crucial to food security for at least half the world population. New varieties with high yield potential, good quality and high resistance to biotic and abiotic stresses are needed in order to meet the demand for more food arising from the rapid human population growth and concurrent decrease in arable land. Improvement of rice quality has now become a foremost consideration for rice buyers and breeding programs.

Quality is defined as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs" (International Standard Organization (ISO) 8402 1986). Features are identified properties of a product which can be related to the quality characteristics. Grain quality of rice is the totality of features and characteristics of rice or rice product that meets the demand of end-user. The concept of grain quality covers many features ranging from physical to biochemical properties, and includes milling efficiency, grain shape and appearance, cooking easiness, eating palatability, and nutrition. Thus, rice grain quality generally includes four classes, i.e. milling quality, appearance quality, cooking and eating quality, and nutritional quality (Figure 1). Many countries have set up their own protocols to assess the respective quality. International organizations such as ISO, Association of Analytical Communities International (AOAC), and American Association of Cereal Chemists International (AACCI) have set up methods to evaluate some quality parameters, for example, apparent amylose content (AAC). Rice is consumed mainly as milled, so eating quality mentioned in this article generally relates to the cooked milled rice. However, due to the impact of the western life style, whole grain rice or brown rice becomes popular worldwide, so that the nutritional quality has expanded to the nutrients of brown rice.

Grain quality and its assessment are not only important to consumers, end-users, processors, but also to rice breeders who are engaged in creating rice varieties harboring new features such as high quality, high yield potential, highly resistant to abiotic or biotic stresses. It is necessary

for rice breeders to understand how the quality traits are inherited from their parents. Genetic studies have revealed many genes and quantitative trait loci (QTL) for grain quality, though the grain quality traits are complex. Some major genes have been cloned, and their functions in a specific pathway, such as starch, protein, lipid, and flavonoids biosynthesis, have been characterized. Some QTLs have been finely mapped for further map-based cloning and functional characterization. The known genes or QTLs have been successfully applied in breeding programs for marker-assisted selection (MAS) to improve the breeding and selection efficiencies.

This chapter highlights the genes and QTLs available for grain quality of rice, summarizing how many QTLs and genes have been mapped or characterized, and how many could be used in marker assisted selection (MAS), which could help breeders to in-deep understand the genetics of grain quality of rice and apply the knowledge in their breeding practices.



Figure 1. Four facets of grain quality

2. Four facets of grain quality

2.1. Milling quality

Milling quality determines the final yield and the broken kernel rate of the milled rice, which is of concern for consumers and farmers. Three main parameters, brown rice recovery (the percentage of brown rice to rough rice), milled rice recovery (the percentage of milled rice to rough rice), and head rice recovery (the percentage of head rice to rough rice) are used to evaluate the quality and efficiency of the milling process. Brown rice is the de-hulled rice with

the palea and lemma removed. Brown rice itself is a type of whole grain that could be used for cooking and eating. Removing all of the bran which consists of the aleurone and pericarp, and germ or embryo from brown rice results in white (or milled) rice. Some milled grains are broken during milling, head rice is a standard term for the whole milled grain. In calculation of head rice recovery, kernels longer than or equal to 3/4 full length of a kernel were considered as whole grains. Among all three parameters to determine the milling quality, head rice recovery is the main factor determining rice market value and one of the most important criteria of milled rice.

2.2. Appearance quality

Appearance is one of the crucial properties of rice grain affecting its market acceptability. After milling, the appearance of the grain is associated with size, shape (long vs. round), chalkiness, and translucency. Grain length, width, thickness are used to describe the physical dimensions of rice kernels, while the grain shape is expressed as the ratio of length to width. Grain appearance is also largely determined the clarity, the vitreousness, and the translucency of the endosperm, which is specifically required by most segments of the rice industry. According to the location of the chalkiness in the endosperm, it could be classified into three groups, white belly (chalkiness on the dorsal side of the grain), white back (chalkiness on the ventral side) and white core (chalkiness in the center). Generally, the great the chalkiness, the lower the market acceptability. Percentage of chalky grain is the proportion of grains having a chalky spot on (or in) the endosperm. Chalkiness is measured visually with scales for 0 for none, 1 for small (<10%), 5 for medium (10-20%) and 9 for large (>20 % of the area). Grain transparency may be measured using a light permeation instrument or with an image analyzer, with which the size and shape may be measured simultaneously.

2.3. Cooking and eating quality

Cooking and eating quality determines the easiness of cooking, as well as the firmness and stickiness of the cooked rice. Rice cooking and eating quality is highly related to some easily measurable physicochemical properties: apparent amylose content (AAC), gel consistency, gelatinization temperature (GT) and pasting viscosity. All these parameters are related to the properties of starch that makes up 90% of milled rice. Starch consists of two kinds of molecules, the linear and helical amylose and the branched amylopectin. Amylose content is measured with a simplified procedure using I₂-KI solution. Due to the binding ability of long chain of amylopectin with I₂, the amylose content measured with I₂-KI solution is also termed as apparent amylose content (AAC). The AAC of milled rice may be classified as waxy (1-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (>25%). Gelatinization is the disruption of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The gelatinization temperature determines the time and energy input required for cooking. Gel consistency was developed as a parameter to index the tendency of cooked rice to harden on cooling, and is normally classified as hard, medium, and soft. Pasting viscosity is another useful parameter to differentiate rice with similar AAC, and is popularly

measured by a Rapid Visco-Analyser (RVA) developed by Newport Scientific Pty Ltd., Australia. RVA records the viscosity continuously as the temperature is increased, held constant for a time, and then decreased.

The above mentioned are objective parameters for the cooking and eating quality. However, eating quality is quite subjective and thus is difficult to define as it depends on consumer preferences. Sensory quality of cooked rice could be evaluated by a trained sensory panel (Champagne et al. 2010). Four steps are used to evaluate the cooked rice texture (Table 1). In addition to texture, the flavor (aromatics, taste, mouthfeel) of cooked rice can also be evaluated by the sensory panel.

Phases/attributes	Definition
Phase I	Place 6–7 grains of rice in mouth behind front teeth. Press tongue over surface and evaluate.
Initial starchy coating	Amount of paste-like thickness perceived on the product before mixing with saliva (3 passes).
Slickness	Maximum ease of passing tongue over the rice surface when saliva starts to mix with sample.
Roughness	Amount of irregularities in the surface of the product.
Stickiness to lips	Degree to which kernels adhere to lips.
Stickiness between grains	Degree to which the kernels adhere to each other.
Phase II	Place 1/2 teaspoon of rice in mouth. Evaluate before or at first bite.
Springiness	Degree grains return to original shape after partial compression.
Cohesiveness	Degree to which the grains deform rather than crumble, crack, or break when biting with molars.
Hardness	Force required to bite through the sample with the molars.
Phase III	Evaluate during chew.
Cohesiveness of mass	Maximum degree to which the sample holds together in a mass while chewing.
Chewiness	Amount of work to chew the sample.
Uniformity of bite	Evenness of force throughout bites to chew.
Moisture absorption	Amount of saliva absorbed by sample during chewing.
Phase IV	Evaluate after swallow.
Residual loose particles	Amount of loose particles in mouth.
Toothpack	Amount of product adhering in/on the teeth.

¹Adapted from Champagne et al. (2010).

Table 1. Sensory descriptive texture attributes and their definitions used to evaluate cooked rice texture¹

2.4. Nutritional quality

As one of the most important staple food in the world, nutritional quality is closely related to human health, and thus is highly valued by consumers. Protein is the second most abundant constituent of milled rice, following starch. Lysine is the first limiting essential amino acid in rice based on the human requirements. Protein and lysine content are two important parameters determining nutritional value of rice. With social development, diverse people eating rice as staple food may require rice with distinct nutritional quality. For those in the underdeveloped region where micronutrient deficiency (Vitamins and minerals, such as iron and zinc) is apparent, genetics study for and biofortification of micronutrients by breeding are necessary to improve the nutritional quality of rice. For those with improved living standards, consuming of brown rice as one kind of whole grains becomes popular to combat chronic diseases, such as diabetes. Whole grain rice (brown rice) provides more minerals, vitamins, dietary fibers, and phenolics to human health than milled rice (Bao 2012a).

3. Genes and QTLs for grain quality

3.1. Milling quality

Milling quality is assessed by brown rice recovery, milled rice recovery and head rice recovery, which is one kind of complex quantitative trait whose genetic control is poorly understood. Up to date, no major gene has been genetically identified and functionally characterized. However, many studies have been carried out to search quantitative trait locus (QTL) for the milling quality (Table 2). These researches improve our understanding of the genetic control of milling quality, and provide molecular markers that are useful in breeding for improvement of milling quality in rice.

Population	Property ¹	No. of QTLs	Chromosome distribution ²	PVE ³	Reference
Brown rice recovery					
Zhenshan 97/Minghui 63	I/I, RIL	1	5	10	Tan et al. 2001
Nipponbare/Kasalath	J/I, BIL	5	3,4,9,10,11	7.5-17.9	Li et al. 2004a
Asominori /IR24	J/I, RIL	2	9, 10	7.2, 21.3	Dong et al. 2004
Caiapo/ <i>O. glaberrima</i>	I/W, DH	3	1, 7, 8	2.8-4.9	Aluko et al. 2004
Zhenshan 97/WYJ-2	I/J, DH	1	12	13.6	Jiang et al. 2005
Teqing/Lemont	I/J, IL	3	5,6,7	5-12.4	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	2	1,3	1.9, 3.2	Lou et al. 2009
L204/01Y110	J/J, RIL	3	1, 4, 6	6-11	Nelson et al. 2012
Milled rice recovery					

Population	Property ¹	No. of QTLs	Chromosome distribution ²	PVE ³	Reference
Zhenshan 97/Minghui 63	I/I, RIL	2	3, 5	4.8, 7.0	Tan et al. 2001
Nipponbare/Kasalath	J/I, BIL	4	4,9,10,11	7.6-19.9	Li et al. 2004a
Asominori /IR24	J/I, RIL	2	11, 12	7.7, 12.2	Dong et al. 2004
Caiapo/ <i>O. glaberrima</i>	I/W, DH	2	5,7	5.3, 6.1	Aluko et al. 2004
Teqing/Lemont	I/J, IL	5	1,2,5,6,7	11.5-30.7	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	1	3	6.7	Lou et al. 2009
L204/01Y110	J/J, RIL	3	1, 4, 9	6-9	Nelson et al. 2012
Head rice recovery					
Zhenshan 97/Minghui 63	I/I, RIL	1	3	10.1	Tan et al. 2001
IR64/ <i>O. rufipogon</i>	I/W, BC2F2	3	1,2,5	5.2-5.5	Septiningsih et al. 2003
Nipponbare/Kasalath	J/I, BIL	3	3, 6,7	9.7-12.2	Li et al. 2004a
Asominori /IR24	J/I, RIL	3	1, 3, 5	8,7-22.1	Dong et al. 2004
Caiapo/ <i>O. glaberrima</i>	I/W, DH	5	1,3,6,8,11	7.6-54.1	Aluko et al. 2004
Zhenshan 97/WYJ-2	I/J, DH	2	3, 8	10.1, 16	Jiang et al. 2005
Teqing/Lemont	I/J, IL	3	1,5,6	5.8-5.9	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	1	3	29.7	Lou et al. 2009
Cypress/RT0034	J/I, RIL	2	6,9	12, 16	Nelson et al. 2011
Cypress/ LaGrue	J/J, RIL	4	1,5,9,10	8, 12	Nelson et al. 2011
L204/01Y110	J/J, RIL	7	6, 6, 8, 9, 9, 10, 11	3-8	Nelson et al. 2012

1: BC=backcross; BIL=backcross inbred line; DH=doubled haploid; I=*indica* subspecies; J=*japonica* subspecies; RIL=recombinant inbred line; W=wild rice. IL: introgression lines.

2: The value in this column indicates the number of chromosome; the two or three same values in the same line indicate two or three QTLs in the same chromosome.

3: Percentage of total variation explained by a single QTL (%).

Table 2. Summary of main-effect QTLs for milling quality traits mapped on rice genome

3.1.1. Brown rice recovery

A total of 20 QTLs have been identified in eight studies, covering all chromosomes except chromosome 2 (Table 2). A major QTL at the interval between markers RM42 and C734b on chromosome 5 is also responsible for grain width (Tan et al. (2001). A QTL on chromosome 3 likely shares the same genomic region for grain length (Lou et al. 2009). These results indicate that brown rice rate relates to the grain shape and size of rice kernel. Five QTLs were detected in the study of Li et al. (2004a), of which three were expressed in two years, indicating that there are QTL-by-environment interactions effects.

3.1.2. Milled rice recovery

A total of 19 QTLs have been identified in seven studies, covering all chromosomes except chromosome 8 (Table 2). There are no strong or reproducible QTLs for the milled rice recovery. Three independent studies detected QTL for the milled rice recovery on chromosome 5 (Tan et al. 2001; Aluko et al. 2004; Zheng et al. 2007), but there are actually not at the same region. Li et al. (2004a) reported that two of four QTLs were detected in two years, indicating that the QTL-by-environment interactions effects exist.

3.1.3. Head rice recovery

Up to date, a total of 34 QTLs locating at all the chromosomes have been reported in ten studies with the number of QTLs varied from 1 to 7 in different studies. A major QTL located on chromosome 3 is also a major QTL for grain length (Tan et al. 2001), suggesting that genetic relationship exists between grain size or shape and the percentage of head rice. Other studies frequently identified the QTL at chromosome 3 (Li et al. 2004a; Dong et al. 2004; Aluko et al. 2004; Jiang et al. 2005; Lou et al. 2009), proving that there might be a major gene for head rice. In addition, QTLs on chromosome 1, 5 and 6 are also detected by at least three independent studies. Li et al. (2004) detected three QTLs for head rice, but all of them were detected only in a specific year, suggesting that the head rice is largely affected by the environment. However, Nelson et al. (2011) showed that more variance of head rice yield was explained by main-effect QTL than QTL \times environment effect in the Cypress/RT0034 RIL population, whereas the main-effect QTLs contributed a little less to genetic variation than those of QTL \times environment effect in the Cypress/ LaGrue RIL population. There is a clear coincidence of QTLs for head rice recovery with early-heading QTLs in the hotter growing location, hinting an environmental effect (Nelson et al. 2011). Note that some genetic populations were derived from cultivated rice and wild rice (Septiningsih et al. 2003; Aluko et al. 2004), but all milling-yield-increasing effects came from the cultivated parent.

3.2. Appearance quality

3.2.1. Grain size and shape

Grain shape is not only key determinant of grain quality but also of grain yield potential. A long, slender grain of rice is generally preferred by consumers in Southern China, the USA, and South and Southeast Asian countries, whereas consumers in Japan, Korea, and Northern China prefer short or round grain of rice (Huang et al. 2013).

Grain length, grain width, length-to-width (grain shape) are the most stable properties of the variety, so they are highly heritable. Genetically, a lot of QTLs have been identified for grain length, grain width, and grain shape (Figure 2a). The chromosome 3 harbors more QTLs than others (Figure 2b). Some are major genes that have been map-based cloned with their function characterized (Table 3), some are finely mapped (Table 4), while many are with minor effects and are waiting for further characterization. The finely mapped QTLs provide potential markers for molecular breeding to modify grain shape while use of functional markers derived

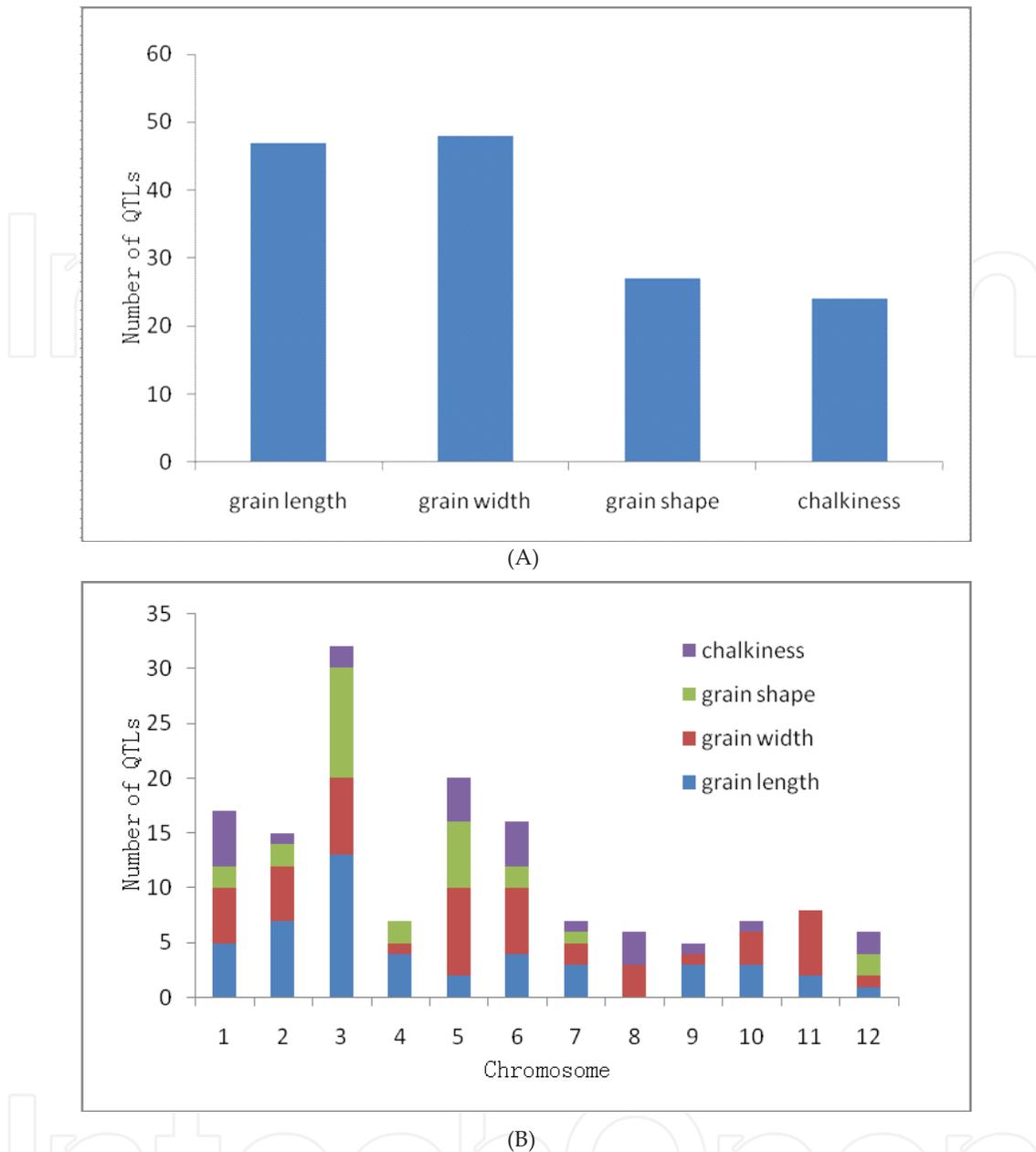


Figure 2. Number (A) and distribution (B) of QTLs for appearance quality (data from Gramene: <http://www.gramene.org/>).

from the cloned genes would lead to precise phenotype in breeding. QTL mapping studies also suggest that many QTLs exhibit pleiotropic effects; they control not only grain length, but also grain width, grain shape or grain yield (Bai et al. 2010; Fan et al. 2006; Guo et al. 2009; Li et al. 2011; Song et al. 2007; Wang et al. 2012). Functional characterization of the cloned genes provides evidence underlying the pleiotropic effects (Fan et al. 2006; Li et al. 2011; Song et al. 2007; Wang et al. 2012). A good review of Huang et al. (2013) has summarized the current progress in the genetic base of grain shape of rice.

Gene	Trait	Chromosome	Encoded protein	Reference
<i>GS3</i>	Grain length	3	Member protein with multiple domains	Fan et al.2006
<i>GL3</i>	Grain length	3	protein phosphatase with Kelch-like repeat domain (OsPPKL1)	Zhang et al. 2012; Hu et al. 2012 Qi et al. 2012
<i>GW2</i>	Grain width	2	Ring-type E3 ubiquitin ligase	Song et al. 2007
<i>GW5/qGW5</i>	Grain width	5	Arginine-rich protein of 144 amino acids	Weng et al.2008; Shomura et al. 2008
<i>GS5</i>	Grain width	5	Serine carboxypeptidase	Li et al. 2011
<i>GW8/SPL16</i>	Grain width	8	SQUAMOSA promoter-binding protein-like 16	Wang et al. 2012
<i>ms-h /UGPase1</i>	grain chalkiness, genic male sterility	9	UDP-glucose pyrophosphorylase 1	Woo et al. (2008)
<i>GIF1</i>	grain chalkiness, grain incomplete filling	4	Cell wall invertase	Wang et al. 2008a

Table 3. The cloned genes for grain appearance quality (grain shape and endosperm chalkiness)

3.2.1.1. Grain length

A total of 47 QTLs for grain length have been detected in different populations. Among them, the Chromosome 3 harbors more QTLs than other chromosomes (Figure 2). Up to date, two QTLs have been map-based cloned (Table 3), and seven QTLs have been finely mapped (Table 4).

GRAIN SIZE 3 (*GS3*) is the first QTL that has been map-based cloned for grain length. It was detected in the RIL population derived from Minghui 63 and Chuan 7, displaying a major role for grain length and weight and a minor role for grain width and thickness and functioning as a negative regulator for grain size (Fan et al. 2006; 2009). The *GS3* protein contains an organ size regulation (OSR) domain in the N terminus, a transmembrane domain, a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR)-like domain, and a von Willebrand factor type C (VWFC) domain in the C terminus. The OSR domain functions as a negative regulator of grain length and deletion mutants of this domain result in the formation of long-grain rice. The C-terminal TNFR/NGFR and VWFC domains act as positive regulators of grain length and loss-of-function mutations of these domains lead to the development of very short grain (Mao et al. 2010; Takano-Kai et al. 2009).

GRAIN LENGTH 3 (*qGL3*) is a major grain length QTL recently identified in three mapping populations (Zhang et al. 2012; Hu et al. 2012; Qi et al. 2012). *qGL3* encodes a putative protein phosphatase with Kelch-like repeat domain (OsPPKL1). A rare allele, i.e a single nucleotide substitution (C → A) leads to a long grain phenotype by an aspartate-to-glutamate transition in

a conserved AVLDT motif of the second Kelch domain in OsPPKL1 (Hu et al. 2012; Zhang et al. 2012; Qi et al. 2012). Genetic analysis of a near-isogenic line (NIL) for qGL3-1 revealed that the allele qGL3-1 from CW23 has an additive or partly dominant effect, and is suitable for use in molecular marker-assisted selection (Hu et al. 2012). A new variety containing the new allele shows increased grain yield, which indicates that GL3 is a powerful tool for breeding high-yield crops (Qi et al. 2012).

3.2.1.2. Grain width

A total of 48 QTLs for grain width have been detected in different populations with more QTLs on chromosome 3 and 5 (Figure 2). Up to date, four QTLs have been map-based cloned (Table 3).

GRAIN WIDTH 2 (GW2) is a major QTL for rice grain width and weight, which was initially detected from a cross between a large-grain japonica rice variety (WY3) and a small-grain *indica* rice variety (Fengaizhan-1). GW2 encodes a RING-type E3 ubiquitin ligase (Song et al. 2007). WY3 has a 1-bp deletion resulting in the introduction of a premature stop codon in its exon 4, causing the large-grain phenotype. GW2 negatively regulates cell division by targeting its substrates to proteasomes for regulated proteolysis; loss of GW2 function results in an increase in cell number in the spikelet hull and acceleration of the grain-milk filling rate, thus enhancing grain width, weight, and yield.

GRAIN WIDTH 5 (GW5) is a major QTL for seed width on chromosome 5 (qSW5) (Wan et al. 2008; Weng et al. 2008; Shomura et al. 2008). A survey of GW5/qSW5 polymorphisms in various rice landraces has revealed that deletions in this gene may have played an important role in the selection of increased grain size from artificial and natural crossings during rice domestication (Shomura et al. 2008). The GW5/qSW5 gene encodes a nuclear protein of 144 amino acids with an arginine-rich domain. Because GW5/qSW5 physically interacts with polyubiquitin, it is likely to act as a regulator in the ubiquitin–proteasome pathway and regulates cell division of the outer glume of the rice spikelet (Wan et al. 2008; Weng et al. 2008; Shomura et al. 2008).

GRAIN SIZE ON CHROMOSOME 5 (GS5) is a major QTL affecting grain width, grain filling, and grain weight (Li et al. 2011). It encodes a serine carboxypeptidase and functions as a positive regulator of grain size. Analysis of genomic DNA sequences and promoter swaps in transgenic plants reveals that nucleotide changes in three segments of the GS5 promoter seem to be responsible for the variations in grain width (Li et al. 2011).

GRAIN WIDTH 8 (GW8) is a major QTL affecting grain width and grain yield from the cross between HXJ74 and Basmati385 (Wang et al. 2012), which encodes SQUAMOSA promoter-binding protein-like 16, referred to OsSPL16, belonging to the protein family of SBP domain-containing transcription factors. Six polymorphisms in the DNA sequence of OsSPL16 exist in the parents HXJ74 and Basmati385. Among them, a 10-bp deletion in the promoter region has been shown to be responsible for the slender grain trait of Basmati385 (Wang et al. 2012).

GS3, GL3, GW2, and GW5/qSW5 are negative regulators of grain size, but GS5 and GW8 are positive regulators of cell proliferation. Other genes associated with grain shape including the SMALL AND ROUND SEED (SRS) loci have been well reviewed in Huang et al. (2013).

Functional markers developed from these major genes and finely mapped QTL resources allow breeders to efficiently manipulate grain size and shape (Tables 3 and 4).

Trait	QTL	Chromosome	Marker interval	Distance ^a	Reference
GL	qGL-3a	3	RMw357–RMw353	87.5kb	Wan et al. 2006
GL	qGL4b	4	RM5586–RM3524	3Mb	Kato et al. 2011
GL/GW	qGL7	7	RID711–RM6389	258kb	Bai et al. 2010
GL	GS7	7	Indel3–Indel5	4.8kb	Shao et al. 2012
GL/GW/GS	qSS7	7	GL293–GL285	23kb	Qiu et al. 2012
GL	qGRL1	1	RM431–CHR1.1	108kb	Singh et al. 2012a
GL	LGS1	2	RM13838–RM13840	0.2cM	Huang et al. 2013
PGWC	qPGWC-8	8	Indel 8G-7-Indel 8G-9	142kb	Guo et al. 2011
PGWC	qPGWC-7	7	Indel14-Indel3 (RM21938)	44kb	Zhou et al. 2009

GL: grain length; GW: grain wideness; GS: grain shape and PGWC: percentage of grains with chalkiness

Table 4. Fine mapped QTLs associated with appearance quality of rice

3.2.2. Grain chalkiness

Chalkiness is a major concern in rice breeding because it is one of the key factors in determining quality and price. The chalky endosperm consists of loosely packed, round and large compound starch granules while the translucent endosperm comprises tightly packed, polyhedral and small single starch granules. The chalky grains show significantly different physicochemical, morphological, thermal, cooking and textural properties from translucent grains. Percentage of grains with chalkiness (PGWC) is one of the main indices of rice-determining appearance quality, which is easily determined.

Many factors contribute to the formation of chalkiness in the rice grain. Environmentally, rice grown at the higher temperature contains more chalky grains. Genetically, defect in genes affecting starch biosynthesis, starch granule structure, and grain filling may lead to endosperm chalkiness. These genes include *starch branching enzyme IIb (BEIIb)*, *branching enzyme I (BEI)*, *starch synthase IIIa (SSIIIa)*, *floury* and *sugary* genes, etc. It should be noted that many of the genes characterized show pleiotropic effects on other traits in addition to chalkiness.

A rice genic male-sterility gene *ms-h* is recessive and has a pleiotropic effect on the chalky endosperm (Woo et al. 2008). Fine mapping and nucleotide sequencing analysis reveal a single nucleotide substitution at the 3'-splice junction of the 14th intron of the UDP-glucose pyrophosphorylase 1 (*UGPase1*) gene, which causes the expression of two mature transcripts with abnormal sizes caused by the aberrant splicing. Overexpression of *UGPase1* in *ms-h* mutant plants restored male fertility and the transformants produced T1 seeds that segregated into normal and chalky endosperms (Woo et al. 2008).

The grain incomplete filling 1 (*gif1*) mutant defects in grain-filling capacity, but its grains are with more chalkiness as a result of loosely packed starch granules. A frameshift mutation caused by a 1-bp nucleotide deletion in *GIF1* results in premature termination of its open reading frame. *GIF1* encodes a cell-wall invertase required for carbon partitioning during early grain filling (Wang et al. 2008a).

Two white-core genes have been characterized with knockout mutants. A floury endosperm-4 (*flo4*) rice mutant with a floury-white endosperm but a normal outer portion was generated by T-DNA insertion into the fifth intron of the *OsPPDKB* gene encoding pyruvate orthophosphate dikinase (PPDK) (Kang et al. 2005). Other two additional alleles, *flo4-2* and *flo4-3* also showed the same white-core endosperm phenotype. *OsPPDKB* was mainly expressed in the endosperm, aleurone, and scutellum of the developing kernel, suggesting that cytosolic PPDK functions in rice to modulate carbon metabolism during grain filling. Ryoo et al. (2007) characterized another white-core floury endosperm mutant (*flo5*) caused by T-DNA insertion into the *SSIIIa*.

A floury mutant, *flo(a)*, exhibits floury characteristics in the innermost endosperm, while the outer layer of the endosperm appeared normal (Qiao et al. 2010). The *FLO(a)* gene was isolated via a map-based cloning approach and predicted to encode the tetratricopeptide repeat domain containing protein, *OsTPR*. Three mutant alleles contain a nucleotide substitution that generated one stop codon or one splice site, respectively, which presumably disrupts the interaction of the functionally conserved TPR motifs (Qiao et al. 2010). The *OsTPR* motifs may play a significant role in rice starch biosynthetic pathways, which causes the formation of chalkiness. Yang et al. (2012) identified a mutant 'Jiangtangdao 1' which had chalky endosperm with resistant starch content up to 11.67%. The putative gene *starch branching enzyme 3* on chromosome 2 was finely mapped and a cleaved amplified polymorphic sequence (CAPS) marker for marker assisted selection was developed (Yang et al. 2012).

For the naturally occurring chalkiness, earlier studies (Li et al. 2004; Tan et al. 2000; Wan et al. 2005) identified 24 QTLs from three crosses among Asian cultivars (Figure 2). Recently, Wan's group in China (Guo et al. 2011; Liu et al. 2010; Wan et al. 2005; Zheng et al. 2012; Zhou et al. 2009) and others (Yamakawa et al. 2008; Liu et al. 2012) have identified many more QTLs for grain chalkiness. Among them, two QTLs have been finely mapped (Table 4).

qPGWC-8 is a major QTL for the percentage of grains with white chalkiness in the interval G1149-R727 on chromosome 8 which was identified using a chromosome segment substitution line (CSSL). Guo et al. (2011) narrowed down the location of this QTL to a 142 kb region between Indel markers 8G-7 and 8G-9. qPGWC-8 accounted for 50.9% of the difference in PGWC between the parents.

qPGWC-7 is a QTL for the percentage of grain with chalkiness (PGWC) on 7 which was identified using a set of chromosome segment substitution lines, made from a cross between PA64s and 9311. Segregation analysis of the F_2 population from the cross between C-51 (a CSSL harboring qPGWC-7 and having a chalky endosperm) and 9311 showed PGWC is a semi-dominant trait, controlled by a single nuclear gene. Fine mapping of qPGWC-7 with a large

F₂ population constructed from the cross C51 × 9311 delimited it to a 44-kb DNA fragment, containing thirteen predicted genes (Zhou et al. 2009).

The markers tightly linked to qPGWC-8 and qPGWC-7 facilitate cloning of the gene underlying the QTLs and is of value for marker-assisted selection for endosperm texture. However, it is still far away from clear understanding the mechanism of formation of the grain chalkiness. First, the QTLs mapping results show low coherence in different genetic populations, suggesting many minor QTLs affecting chalkiness exist in different rice germplasm that we do not know. Second, in addition to the major genes or QTLs we have known, how their interactions with each other, and with the major genes for amylose and protein synthesis (Liu et al. 2010; Zheng et al. 2012) that may affect chalkiness are unknown. Third, effect of environment on the formation of chalkiness is well known, but how its effect on the gene expression that leads to the formation of chalkiness is largely unknown.

3.3. Eating and cooking quality

Great progresses have been made in the understanding of the genetic basis of cooking and eating quality (Bao 2012b; Chen et al. 2012). Starch properties play important role in determining the cooking and eating quality, which is highly associated with starch biosynthesis related genes. Starch biosynthesis pathways and genes or enzymes participating in have been well clarified (Figure 3). Amylose is synthesized mainly by GBSSI, and the amylopectin synthesis process is governed by a combination of multiple isoforms of SS, BE, and DBE to produce a uniform number of chains per amylopectin cluster. *Wx* encoding GBSSI is mainly responsible for the natural variation of amylose content, gel consistency and RVA pasting viscosity, while the *SSIa* is mainly for gelatinization temperature, thermal properties, and amylopectin structure (Bao 2012b).

3.3.1. Apparent amylose content, gel consistency and RVA pasting viscosity

Wx locus on chromosome 6 is a major QTL for amylose content, gel consistency and RVA pasting viscosity (He et al. 1999; Bao et al. 2000; Bao 2012; Wan et al. 2004; Fan et al. 2005; Septiningsih et al. 2003; Aluko et al. 2004; Lapitan et al. 2009; Lanceras et al. 2000; Tan et al. 1999). Map-based cloning of the qGC-6, a locus for gel consistency, indicates that *Wx* is the major gene controlling it (Su et al. 2011). Five functional markers in the *Wx* gene, a (CT)_n microsatellite (or simple sequence repeat, Ayres et al. 1997; Bligh et al. 1995;), a 23bp insertion/deletion sequence (Inukai et al. 2000; Wanchana et al. 2003; Teng et al. 2012), and three single nucleotide polymorphism (SNP) markers (Bligh et al. 1998; Cai et al. 1998; Hirano et al. 1998; Isshiki et al. 1998; Larkin and Park 2003) are well characterized, with different alleles differing in AAC (Ayres et al. 1997; Bligh et al. 1995; Chen et al. 2008a; Inukai et al. 2000; Larkin and Park 2003), and RVA pasting viscosity (Bao et al. 2006a; Chen et al. 2008b; Larkin et al. 2003; Larkin and Park 2003). Among them, the (CT)_n microsatellite in the *Wx* gene located 55 bp upstream of the putative 5'-leader intron splice site has many alleles with *n* ranging from 8 to 22 in diverse rice germplasm (Ayres et al. 1997; Bergman et al. 2001; Bao et al. 2006a; Chen et al. 2008a; Bao et al. 2002a; Han et al. 2004). Another locus, the G/T single nucleotide polymorphism (SNP) at the putative leader intron 5' splice site, and a G to T mutation at this site reduces

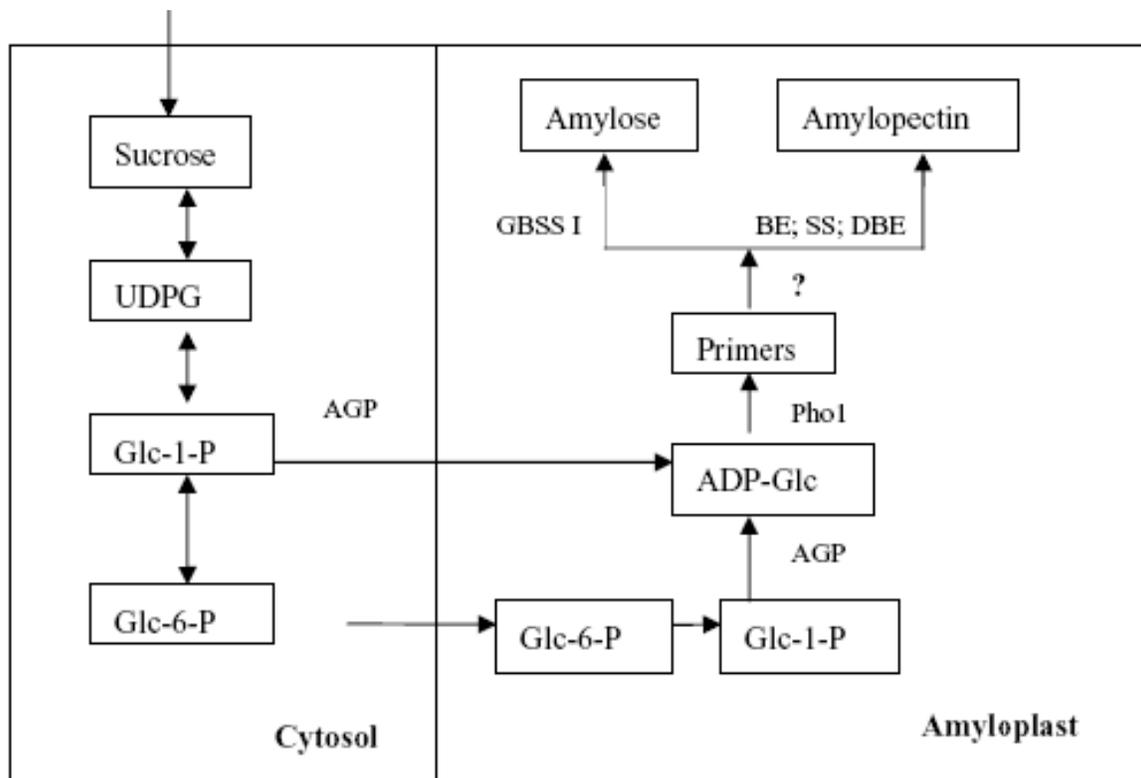


Figure 3. Starch biosynthesis pathway in rice endosperm (modified from Jeon et al. 2010). Starch biosynthesis consists of two distinct phases: the glucan initiation process and the starch amplification process. The plastidial starch phosphorylase (Pho1) extends the chains of the initial priming sites such as free chains of malto-oligosaccharides in the presence of Glc-1-P. The subsequent mechanisms underlying the glucan initiation process remain to be established. Branched dextrans are putatively processed by the coordinated activities of SS, BE, and/or DBE to produce the prototype of an amylopectin cluster structure, which further develops into amylopectin to establish the basic structure. AG-Pase, ADP glucose pyrophosphorylase; BE, starch branching enzyme; DBE, starch debranching enzyme; GBSSI, granule-bound starch synthase; Pho1, plastidial starch phosphorylase; SS, soluble starch synthase; DBE includes isoamylase (ISA) and pullulanase (PUL).

the efficiency of *Wx* pre-mRNA processing and thus results in the lower level of spliced mature mRNA, *Wx* protein, and AAC (Wang et al. 1995; Bligh et al. 1998; Cai et al. 1998; Hirano et al. 1998; Isshiki et al. 1998). Waxy, low amylose, and some intermediate amylose rice have the T SNP allele, while some intermediate and high amylose rices have the G allele (Ayres et al. 1997; Bligh et al. 1998; Cai et al. 1998; Isshiki et al. 1998). The G/T SNP explained 80% (Ayres et al. 1997) to 90 % (Bao et al. 2006a) of the total observed variation in AAC in the nonwaxy rice accessions.

Rice with similarly high AAC still differs in cooking and eating quality due to potential effect of amylopectin structure and other factors. Gel consistency and RVA pasting viscosity are effective to differentiate rice with high AAC. Genetic studies show that the exon 10 SNP of *Wx* is responsible for the genetic basis for the gel consistency, the proportion of amylose bound to amylopectin, the proportion of amylose able to leach, gel hardness (Tran et al. 2011) and RVA pasting viscosity (Traore et al. 2011). Tran et al. (2011) indicated that the rice with SNP allele C at exon 10 produces soft, viscous gels, has a soft texture when cooked, but with high

retrogradation, and the rice with SNP allele T gives a short, firm gel, and has a firm texture when freshly cooked with little change in texture over storage. In a cross between two varieties having similar high AAC, but with different paste viscosity properties, Traore et al. (2011) indicated that the exon 10 SNP marker is associated with most RVA pasting measurements and the proportion of soluble to insoluble apparent amylose.

3.3.2. Gelatinization temperature, thermal properties

SSIIa locus on chromosome 6 is a major QTL for gelatinization temperature and amylopectin structure (Aluko et al. 2004; He et al., 1999; Bao et al., 2004; Fan et al., 2005; Wang et al. 2007; Tian et al. 2005; Lapitan et al. 2009; Umemoto et al. 2002). Map-based cloning of the alkali degenerate locus gives evidence that the gene encoding SSIIa is the major gene responsible for gelatinization temperature (Gao et al. 2003). Nakamura et al. (2005) revealed that the function of SSIIa is to elongate the short A and B1 chains with degree of polymerization (DP) < 10 to form long B1 chains of amylopectin. Genetic engineering by introduction of *indica* active *SSIIa* gene into *japonica* rice increases GT and gives longer amylopectin side chain length (Nakamura et al. 2005; Gao et al. 2011).

Four functional SNPs in the SSIIa gene have been revealed (Umemoto et al. 2004; Umemoto and Aoki 2005; Nakamura et al. 2005; Bao et al. 2006b; Waters et al. 2006). The first one is at 264 bp in Exon 1 of AY423717, where a change from G to C results in change of glutamate to aspartate. The second site is at 3799 bp, where glycine encoded by GGC is replaced by serine encoded by AGC. The third site is at 4198 bp, where valine encoded by GTG is replaced by methionine encoded by ATG. The fourth site is at 4330 bp, glycine-leucine encoded by GGGCTC is replaced by glycine-phenylalanine encoded by GGTTTC. *SSIIa* gene fragments shuffling experiments by Nakamura et al. (2005) show that only the third and fourth SNPs are functional, and the third SNP (G/A) is crucial for SSIIa activity, the enzyme is inactive when it is A SNP (coding for methionine) no matter which SNP at 4229/4330 bp (GC/TT) is present. The GC/TT is most common and is strongly associated with GT (Waters et al 2006; Bao et al. 2006b). This GC/TT polymorphism alone can differentiate rice with high or intermediate GT (possessing the GC allele) from those with low GT (possessing the TT allele), explaining 62.4 % of the total variation in pasting temperature (Bao et al. 2006b). Few rice accessions with GC allele have low GT phenotype, which can be explained by their carrying the A SNP allele in the third SNP (Umemoto et al. 2004; Waters et al. 2006; Lu et al. 2010). However, it should be mentioned that the A allele of third SNP (G/A) is quite rare in natural populations. The frequency of A at is 1 in 30 rices (Bao et al. 2006b), 9 in 180 rices (Chen et al. 2003), 127 in 1543 rices (Cuevas et al. 2010), 5 in 65 rices (Umemoto et al. 2004), and 13 in 73 rices (Waters et al. 2005). It should also be noted that genetic control of intermediate GT rice starch remains unknown. Intermediate GT rice is characterized by more chains of DP24-35, which may be synthesized by other enzymes (Cuevas et al. 2010).

3.3.3. Contributions of other starch biosynthesis related genes

Cooking and eating quality is a complex trait which is not only determined by the *Wx* and *SSIIa* genes, but also other genetic factors, such as other starch biosynthesis related genes. Three

evidences show the effect of other genes in determining the cooking and eating qualities. First, In a population derived from two parents having similar intermediate AAC, QTLs rather than *Wx* locus are associated with the RVA pasting viscosities, and two of which might be located close to the *starch branching enzyme 1 (SBE 1)* and *SBE3* loci (Bao et al. 2002b). Second, in an association mapping with all the starch biosynthesizing genes, additional five genes (*AGPlar*, *PUL*, *SSI*, *SSIIa*, and *SSIII-2*) with minor effects were detected when the effect of *Wx* gene was eliminated. Again, with the model controlling for *SSIIa*, a further search identified *Wx*, *SBE3*, *ISA*, and *SSIV-2* as minor genes that affect GT additively (Tian et al. 2009). Third, what factors will determine the cooking and eating quality of waxy rice is complex, because the GBSS is not active in waxy rice. It is expected that genes other than *Wx* are to control the genetic basis of pasting and thermal properties of waxy rice. Comparing starch physicochemical properties among different microsatellite groups in *starch branching enzyme 1 (SBE1)* and soluble starch synthase 1 (*SSS*), waxy rices with the *SBE-A* allele have higher peak viscosity (PV), hot paste viscosity (HPV) and cold paste viscosity (CPV) than those with other alleles, and those with the *SSS-B* allele have higher HPV and CPV than other alleles (Bao et al. 2002a). Han et al. (2004) indicated that nucleotide polymorphisms in both *SBE1* and *SBE3* loci account for 70% of the observed variation in HPV and CPV, and for 40% of the observed variation in PV. Yan et al. (2011) conducted association analysis for pasting viscosity parameters of waxy rice using starch synthesis-related gene markers, showing that 10 gene markers were involved in controlling the pasting viscosity parameters. Among these, the *pullulanase* gene plays an important role in control of PV, HPV, CPV, breakdown viscosity, peak time, and pasting temperature (PT) in glutinous rice.

To date, there are many markers resources derived from starch biosynthesis related genes available for molecular breeding for the purpose of improving the cooking and eating quality (Tian et al. 2010; Bao et al. 2006b; Jin et al. 2010; He et al. 2006; Yan et al. 2011).

3.3.4 Other traits related to cooking and eating quality

In addition to the amylose content, gelatinization temperature, gel consistency and pasting viscosity, other parameters, such as water absorption, volume expansion and cooked rice elongation have been set up to evaluate the cooking characteristics of rice (Bao et al. 2009).

Ahn et al. (1993) identified a QTL on chromosome 8 for cooked rice elongation. Rani (2011) found that a functional marker targeting an SNP in the *GS3* is associated with kernel elongation. Tian et al. (2005) detected 3, 2, and 2 QTLs for water absorption, volume expansion and cooked rice elongation, respectively in a DH population. While no QTL on chromosome 3 and 8 was detected, one common QTL for all the traits is at the *Wx* locus on chromosome 6, suggesting that the *Wx* gene plays a major role in determining these cooking characteristics in addition to other cooking and eating quality traits (Tian et al. 2005).

The aroma of cooked rice contributes to consumer sensory acceptance of rice. The aromatic compound 2-acetyl-1-pyrroline (2-AP) reportedly is the primary component of the popcorn-like smell of aromatic rice. Fragrance (*fgr*) is a recessive trait that is controlled by a major gene on chromosome 8 (Lorieux et al. 1996; Jin et al. 2003). Bradbury et al. (2005a; 2005b) reported that the *badh2* gene could most likely be the *fgr* gene since it has an 8-bp deletion and three

SNPs in its exon 7 compared to the functional *Badh2* gene which encodes putative betaine aldehyde dehydrogenase 2 (BADH2), and developed molecular markers for fragrance genotyping. Shi et al., (2008) found a novel null *badh2* allele (*badh2-E2*), which has a sequence identical to that of the *Badh2* allele in exon 7, but with a 7-bp deletion in exon 2. By map-based cloning strategy, Chen et al. (2008c) confirmed that the full-length BADH2 protein encoded by *Badh2* renders rice nonfragrant by inhibiting biosynthesis of 2-acetyl-1-pyrroline (2AP), a potent flavor component in rice fragrance. Functional markers derived from *fgr* are sufficient to carry out molecular marker assisted breeding to improve the sensory quality of rice (Shi et al. 2008; Chen et al. 2008c; Jin et al. 2010). So far as we are aware, there is no genetic report on the other sensory characteristics of rice.

3.4. Nutritional quality

Few molecular genetics studies have been conducted for nutritional quality (Table 5), but many molecular breeding activities through transgenic engineering to improve nutritional quality of rice have been reported (see 4.4).

3.4.1. Protein and amino acid content

There are nice reports about QTL mapping for protein content (Table 5). A total of 43 QTLs have been identified covering all 12 chromosomes. Chromosomes 1, 2 and 7 harbor more QTLs than other chromosomes. In addition to the total protein content, Zhang et al. (2008) detected 2, 4, 3 and 4 QTLs for protein fractions, albumin, globulin, prolamin and glutelin, respectively. The QTLs affecting contents of different protein fractions may locate at the same chromosomal region.

Wang et al. (2008c) identified 18 chromosomal regions for 19 individual amino acids, one of which at the bottom of chromosome 1 is a relatively strong QTL cluster, consisting of up to 19 individual QTL. A wide coincidence was found between the QTL and the loci involved in amino acid metabolism pathways, including N assimilation and transfer, and amino acid or protein biosynthesis (Wang et al. 2008c). Hu et al. (2009) identified a total of 12 QTLs for individual amino acid content and total amino acid content on chromosomes 1, 4, 6, 7 and 11. A QTL cluster on chromosome 1 was associated with the content of eight amino acids. The results are useful for candidate gene identification and marker-assisted breeding targeting the development of improved rice amino acid composition for human nutrition.

3.4.2. Fat content

Fat content affects eating quality and nutritional values, and storage stability of rice as well. Apparently, 48 QTLs for fat content have been reported. Chromosome 1, 3 and 6 harbor more QTLs than other chromosomes (Table 5). Liu et al (2009) reported 14 QTLs for crude fat content in brown rice distributing on chromosomes 1, 3, and 5-9. One of which is a major QTL, qCFC5, locating on chromosome 5, which have been detected simultaneously among three populations. Shen et al. (2012) characterized two stably expressed QTLs on chromosome 7, and they were detected in all three environments and were further confirmed by additional lines across

Population	Property ¹	No. of QTLs	Chromosome distribution ²	PVE ³	Reference
Protein content					
Koshihikari/Kasalath	J/I, BIC	7	2,2,3,4,7,7,10	4.5-15.8	Zheng et al. 2012
Asominori/IR24	J/I, RIL	3	1,3,8	8.5-13.9	Zheng et al. 2011
Zhenshan 97B/Delong 208.	I/I, RIL	6	1,2,4,7,8,9	4-25.9	Zhong et al. 2011
Asominori/IR24	J/I, CSSL	8	1,1,2,3,6,8,8,11	3-54	Liu et al. 2011
Xieqingzao B/Milyang 46	I/I, RIL	5	3,4,5,6,10	4-19	Yu et al. 2009
Asominori/IR24	J/I, RIL	3	2, 7, 12	11-14	Zhang et al. 2008
Gui 630/02428	I/J, DH	5	1,4,5,6,7,	7-35	Hu et al. 2004
Zhenshan 97B/Minghui 63	I/I, RIL	2	6,7	6, 13	Tan et al. 2001
Caiapo/ <i>O. glaberrima</i>	I/W, DH	4	1,2,6,11	3.3-5.8	Aluko et al. 2004
Fat content					
Fengaizhan-1/JZ1560	I/J, F2; F2:3	5	1,3,7,8,10	7.7-13.9	Ying et al. 2012
Samgang/Nagdong	J/I, DH and RIL	8	1,1,3,5,6,6,7,9	6-19	Qin et al. 2010
Sasanishiki/Habataki	J/I, BC	7	1, 2, 3, 6 10, 11,12	5-21	Shen et al. 2010
Xieqingzao B/Milyang 46	I/I, RIL	4	3,5,6,8	7-13	Yu et al. 2009
Zhenshan 97B/Wuyujing 2	I/J, DH and BC1F1	10	1,1,3,3,5,6,7,7,8,9	3.8-21.3	Liu et al. 2009
Asominori/IR24	J/I, RIL	11	1,1,2,3,3,4,4,5,6,6,11	7-14	Wang et al. 2008b
Gui 630/02428	I/J, DH	3	1,2,5	7.7-25.5	Hu et al. 2004

1: BC=backcross; BIL=backcross inbred line; DH=doubled haploid; I=*indica* subspecies; J=*japonica* subspecies; RIL=recombinant inbred line; W=wild rice. IL: introgression lines.

2: The value in this column indicates chromosome number, the two or three same values in the same line indicate two or three QTLs in the same chromosome.

3: Percentage of total variation explained by a single QTL (%).

Table 5. QTLs for protein content and fat content in the rice grain

six environments. The stably expressed QTLs and major QTLs are suitable candidates for the improvement of FC via marker assisted breeding. Dynamic expression of QTLs for fat content during grain filling was detected by Wang et al. (2008b). Eleven unconditional QTL and 10 conditional QTL for FC were identified with more QTL expressed in the early developmental stages. The results suggested that accumulation of fat was governed by time-dependent gene expression. Ying et al. (2012) identified QTLs for fatty acid composition, and 29 associated QTLs were identified throughout the rice genome, except chromosomes 9 and 10. Nine rice

orthologs of *Arabidopsis* genes encoding key enzymes in lipid metabolism co-localized with 11 mapped QTLs. A strong QTL for oleic (18:1) and linoleic (18:2) acid is associated with a gene encoding acyl-CoA:diacylglycerol acyltransferase, while another one for palmitic acid (16:0) is possibly associated with the acyl-ACP thioesterase gene.

3.4.3. Minerals

Stangoulis et al. (2007) mapped the QTLs for inorganic phosphorus (P), total P, Fe, Zn, Cu and Mn concentrations. Norton et al. (2010) mapped 41 QTLs for the concentration of 17 elements in rice grain. Du et al. (2013) identified 23 and 9 QTLs for Ca, Fe, K, Mg, Mn, P, and Zn contents in brown rice in two environments of China, Lingshui of Hainan and Hangzhou of Zhejiang, respectively. Only 2 QTLs for Mg accumulation have been detected in both environments, indicating that mineral accumulation QTLs in rice grains are largely environment-dependent. Garcia-Oliveira et al. (2009) identified 31 putative QTLs for Fe, Zn, Mn, Cu, Ca, Mg, P and K contents with introgression lines derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipogon*). It was found that wild rice contributed favorable alleles for most of the QTLs (26 QTLs), and chromosomes 1, 9 and 12 exhibited 14 QTLs (45%) for these traits.

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) in rice grain may form complexes with mineral ions, such as Fe, Zn and Ca, leading to be low bioavailability of minerals to humans. A set of low phytic acid rice mutant lines with the aim of increasing the bioavailability of the minerals of rice (Liu et al. 2007) have been isolated. Functional markers have been developed from some mutants (Zhao et al. 2008; Tan et al. 2013), and candidate genes such as multi-drug resistance-associated protein ABC transporter gene 5 (Xu et al. 2009) have been revealed. These mutant and markers tagged for the mutation may help develop new rice with increased mineral bioavailability.

3.4.4. Phenolics

Jin et al. (2009) found via linkage mapping that phenolic content, flavonoid content, and antioxidant capacity were individually controlled by three QTLs. Only one QTL on chromosome 2 was shared by phenolic content and flavonoid content. Shao et al. (2011) identified QTLs for these traits via association mapping using a diverse set of rice germplasm including red rice and black rice. Four, six and six QTLs were found associated with phenolic content, flavonoid content, and antioxidant capacity, respectively. Among them, four QTLs for phenolic content were also shared for other two traits. *Ra* (i.e. *Prp-b* for purple pericarp) and *Rc* (brown pericarp and seed coat) were main-effect loci for rice grain color and nutritional quality traits. Association mapping for the traits of the 361 white or non-pigmented rice accessions (i.e. excluding the red and black rice) revealed marker (RM346) is associated with phenolic content.

Pigmented rice accumulates anthocyanins (black rice) and proanthocyanidin (red rice), which are benefit to human health. Genetically, the pericarp color of red rice was controlled by two complementary genes, *Rc* (brown pericarp) on chromosome 7 and *Rd* (red pericarp) on

chromosome 1. When present together, these loci produce red seed color. *Rc* in the absence of *Rd* produces brown seeds, whereas *Rd* alone has no phenotype (Sweeney et al. 2006; Furukawa et al. 2007). A natural mutation in *rc* has reverted brown pericarp to red pericarp and resulted in a new, dominant, wild-type allele, *Rc-g* (Brooks et al. 2008). The color of dark purple pericarp was also controlled by two complementary genes, *Pb* and *Pp*, located on chromosome 4 and 1, respectively (Wang et al 2009). Wang and Shu (2007) mapped *Pb* gene and suggested that this gene may be *Ra* gene. Markers for these genes may be useful for pigmented rice breeding, especially useful if new rice expects to accumulate both anthocyanins and proanthocyanidin.

4. Molecular breeding

Molecular breeding is the application of molecular biology tools in plant breeding, which generally include marker assisted selection (MAS) and genetic engineering (genetic transformation) in addition to QTL mapping or gene discovery. Both of MAS and genetic engineering have been applied in grain quality improvement in rice. MAS has been successfully applied for cooking and eating quality improvement because of available of the excellent markers, while the genetic engineering has been widely used to improve nutritional quality of rice.

4.1. Marker assisted selection

QTLs underlying natural occurring variation in grain quality have been widely explored, however, only few of them have been applied in current rice breeding programs. To the best of our knowledge, most of reports in terms of improving grain quality simply mean to improve the eating and cooking quality. The most useful genes are *Wx*, *SSIIa*, and *fragrance* (Table 6). Functional markers developed from GS3 are also available for grain length improvement (Wang et al. 2011). There are two strategies to conduct MAS in the breeding program. One is to improve the grain quality for the rice with high yield potential or high resistance to abiotic or biotic stresses, but with low quality. This is referred to foreground selection, which means that selection of a marker for grain quality trait by MAS denotes a trait obtained. Foreground selection is particularly useful for traits that need laborious or time-consuming phenotypic screening procedures, such as grain quality traits. The other is to improve the yield potential and high resistance for good quality rice, such as basmati or jasmine rice. This is referred to background selection. The markers for grain quality are used as background selection, which is to avoid the loss of good quality traits during introduction of the other traits.

4.1.1. *Wx*, *fgr* and *SSIIa*

Low quality of hybrid rice in China is mainly owing to its poor quality maintainer line. One of good ways is to improve the quality of maintainer line by MAS. Some important maintainer line, such as Zhenshan 97B (Zhou et al. 2003; Liu et al. 2006), Longtefu B (Liu et al. 2006), and II32B (Jin et al. 2010), G46B (Gao et al. 2009) have been the target of transferring the *Wx* allele conferring lower amylose content. The new hybrid rice derived from the improved maintainer line and restorer line is expected to have better quality

because the restorer line generally has good quality. Furthermore, MAS with *Wx* gene marker for quality improvement of the conventional rice has been reported (Yi et al. 2009; Jantaboon et al. 2011; Jairin et al. 2009).

Consumers generally prefer fragrant rice to non-fragrant rice. Functional markers for *fgr* have been developed and successively used to transfer this gene from fragrance rice to the target non-fragrance rice (Yi et al. 2009; Jin et al. 2010; Salgotra et al. 2012; Jantaboon et al. 2011).

SSIIa is responsible for the variation of gelatinization temperature; the functional markers for SSIIa have been developed and used in MAS to improve the cooking quality (Jin et al. 2010; Jantaboon et al. 2011; Lu et al. 2010).

Gene marker	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
<i>Wx</i>	484: ctttgtctatctcaagacac	485: ttgcagatgttctctctgatg	Aryes et al. (1997)
<i>SSIIa</i>	NF1 :cgaggcgcagcacaacag F22: caaggagagctggagggggc	NR1: ggccgtgcagatcttaaccat R21: acatgccgcgcacctggaaa	Bao et al. (2006b) and Jin et al. (2010)
<i>fgr</i>	1F : ggagcttgctgatgtgtgtaaa 2F : cctctgctctgcctctgat	1R : ggaacaacctaaccatag 2R : gattgcgcggaggacttg	Jin et al. (2010) Shi et al. (2008)

¹Adapted from Jin et al. (2010)

Table 6. Useful PCR markers for MAS to improve cooking and eating quality of rice¹

4.1.2. Combining grain quality with other traits

Breeding is working for not only one trait, but all the traits for the formation of a new variety. In addition to grain quality traits, yield and other agronomic or resistance traits are also very important. For those rice cultivars already have good quality, the objective of MAS is to combine the important quality traits with other traits. There are special cases for basmati and jasmine rices which have premium grain quality, and have been widely accepted by consumers worldwide. MAS has been carried out to introduce bacterial blight resistance (Pandey et al. 2013; Win et al. 2012), blast resistance (Singh et al. 2012), brown planthopper resistance (Jairin et al. 2009), submergence tolerance (Jantaboon et al. 2011) and plant stature (Pandey et al. 2013) genes into the basmati or jasmine rices.

4.2. Transgenic engineering

The advantage to conduct MAS is that abundant molecular markers are available for rice and many traits have been tagged with molecular markers. However, the disadvantage is that MAS is only effective when the target traits exist in rice germplasm, and becomes void when the traits of interest are not present in the rice germplasm. In this case, transgenic engineering is useful, which could introduce the new traits into rice by transferring the target gene from other species. Expression of exotic gene in rice could produce the target trait. Transgenic engineering

has some successful examples to introduce new nutrient traits into rice grain, such as vitamin A (Va), that confers rice high nutritional and increased benefit to human health.

4.2.1. Resistant starch

Consumption of resistant starch enriched foods is associated with decrease in the postprandial glycaemic and insulinaemic responses, accompanied by the production of fermentation-related gases in the large bowel. A high-amylose transgenic rice line modified by antisense RNA inhibition of starch branching enzymes has a 8.05% of resistant starch content, which was shown to decrease the postprandial glycaemic and insulinaemic responses and promoted fermentation-related production of H₂ in the large bowel of young and healthy adults who consumed the resistant starch-enriched rice meal (Li et al 2009).

4.2.2. Protein

Expression of a gene encoding a precursor polypeptide of sesame 2S albumin, a sulfur-rich seed storage protein in transgenic rice plants results in the improvement of the nutritive value of rice; the crude protein content in rice grains was increased by 0.64-3.54%, and the methionine and cysteine contents of these transgenic rice grains were respectively elevated by 29-76% and 31-75% compared with those of wild-type rice grains (Lee et al. 2003). Over-expression of aspartate aminotransferase genes in rice results in altered nitrogen metabolism and increased amino acid content and protein contents in seeds (Zhou et al. 2009).

4.2.3. Va

Vitamin A deficiency has been linked to night blindness, corneal scarring and permanent blindness. Vitamin A deficiency increases infant mortality rates and the incidence and severity of infectious diseases. Carotenoids, a precursor of Vitamin A, is an important lipid-soluble antioxidants in photosynthetic tissues, which are known to be completely absent in rice endosperm. The entire β -carotene biosynthetic pathway in rice endosperm has been introduced into rice by transformation of plant phytoene synthase, *Erwinia uredovora* carotene desaturase, and lycopene β -cyclase genes via *Agrobacterium*-mediated transformation. The transgenic rice, Golden Rice 1, can accumulate a maximal level of 1.6 $\mu\text{g/g}$ total carotene in the endosperm. Insertion of the phytoene synthetase gene from maize and the carotene desaturase gene from *Erwinia uredovora* into rice resulted in the greatest accumulation of total carotenoids and β -carotene. Golden Rice 2 contains as much as 37 μg total carotenoids per gram of dry weight of grain, of which 31 $\mu\text{g/g}$ is β -carotene (Paine et al. 2005).

4.2.4. Folate

Folates are B vitamins (vitamin B9). Humans cannot synthesize folates and have to absorb them from the diet, with plants usually being the main dietary sources. Folates play roles in the prevention of neural tube defects and in reducing the risk of cardiovascular disease, colon cancer, and neuropsychiatric disorders. In the United States, folic acid is added to refined cereals and grain products; these products are major contributors to total folate intake. Rice is

a poor source of folates (vitamin B9). Overexpressing two *Arabidopsis thaliana* genes of the pterin and para-aminobenzoate branches of the folate biosynthetic pathway, Storozhenko (2007) obtained transgenic rice with a maximal folate content enhancement as high as 100 times above wild type, with 100 g of polished raw grains containing up to four times the adult daily folate requirement.

4.2.5. Minerals (Fe)

Iron deficiency is the most widespread micronutrient deficiency world-wide that afflicts an estimated 30% of the world population, especially where vegetable-based diets are the primary food source. Expression of the soybean *ferritin* gene (Goto et al. 1999) or pea *ferritin* gene (Ye et al. 2007) in rice produced seeds with greater Fe contents. Especially, Vasconcelos et al. (2003) showed that expression of the soybean *ferritin* gene under the control of the glutelin promoter in rice has proven to be effective in enhancing grain nutritional levels, not only in brown grains but also in polished grains. Expression of a thermotolerant phytase gene from *Aspergillus fumigatus* in rice endosperm is expected to decrease the phytic acid and increase iron bioavailability (Lucca et al. 2001).

4.2.6. Flavonoids

Flavonoids are lacking in the endosperm of rice. Expression of maize C1 and R-S regulatory genes driven by an endosperm specific promoter of a rice prolamin gene in rice grain resulted in dark brown pericarp of the C1/R-S homozygous lines, and the major flavonoids, dihydroquercetin (taxifolin), dihydroisorhamnetin (3'-O-methyl taxifolin) and 3'-O-methyl quercetin were identified in the rice grain (Shin et al. 2006). These rice lines have the potential to be developed further as a novel variety that can produce various flavonoids in its endosperm.

4.2.7. Serotonin

Serotonin derivatives such as *p*-coumaroylserotonin and feruloylserotonin, a family of plant polyphenol compounds, play roles in an array of biological activities including antioxidative activity, but neither their production nor identification has been reported in crop plants. Transgenic rice expressing the pepper hydroxycinnamoyl-CoA:serotonin N-(hydroxycinnamoyl) transferase gene produced on average 274 ng/g seed weight which was nine-fold higher than wild-type (30 ng/g seed weight) (Kang et al. 2005). Chemical treatments such as trans-cinnamic acid and tyramine increased the serotonin derivatives contents by two- to three fold in both wild-type and transgenic rice. The transgenic rice had higher radical scavenging activities than that of wild-type, suggesting that nutraceutical serotonin derivative could be enriched by transgenic engineering (Kang et al. 2005).

4.2.8. Coenzyme Q

Coenzyme Q (CoQ), also called ubiquinone, is an electron transfer molecule in the respiratory chain. CoQ is also a lipid-soluble antioxidant. Most cereal crops produce mainly CoQ9, which has nine isoprene units, whereas humans produce mainly CoQ10, with 10 isoprene units.

CoQ10 is a very popular food supplement. Takahashi et al. (2009) produced CoQ10-enriched rice plants by introduction of the gene for decaprenyl diphosphate synthase. In CoQ10-enriched rice plants, seed CoQ10 content per weight was increased to up to 10 times that of wild-type rice, but its level is still insufficient for practical use. Combination of the transgene with giant embryo mutant lines produced giant embryo line-type CoQ10-enriched rice with seed CoQ10 content per weight increased to up to 1.4-1.8 times. It was found that CoQ was preferentially accumulated in bran and germ of rice seed.

5. Future directions

Great progress has been achieved in our understanding of the genetic and molecular basis of grain quality of rice. This is especially true for grain appearance and grain shape, since they are not only linked with grain quality, but also with grain yield, a more important trait. Cooking and eating quality has a strong relation with starch biosynthesis pathway which has been well understood. Markers derived from the starch biosynthesis related genes have been widely applied in MAS. However, there are four major problem areas that challenge researchers working on molecular genetics of grain quality.

5.1. Functional genes for milling quality and chalkiness

Genetic understanding of milling quality is quite poor since only limited numbers of QTLs have been detected, and no QTL has been finely mapped or cloned. To make in-deep research into the area of milling quality, (1) rapid and accurate analytical tools are needed to measure the trait; (2) finely dissection of QTLs with large effect should be carried out; (3) because no mutants for milling quality have been reported, the mutants such as those induced by T-DNA insertion may provide a good start to characterize the genes responsible for milling quality. For grain chalkiness, two finely mapped QTLs await further characterization, and transcriptome for chalkiness formation during seed development have been described (Yamakawa et al. 2007; Liu et al. 2010). It looks optimism to see more progress from this area.

5.2. Molecular genetics studies for nutritional quality

Nutrition quality of rice will be a new area for further research because people keep increasingly concern about the health benefit of the food they eat. Nutrition quality covers a wide range of traits, for example, protein, amino acids, fat and phenolics. In this area, naturally occurring variation for protein, amino acids, fat and fatty acid compositions have been under exploration, but only few genes have been characterized. Formation of each nutrient in rice grain requires a complex pathway in which many genes or enzymes are involved. Current advances in protein and fatty acid biosynthesis in other crops and *Arabidopsis* may help understand the pathways in rice.

Phenolics are expected to be an important field because they are proven to benefit human health in many ways (Shao and Bao 2012). Genes for red pericarp formation, *Rc* (brown pericarp) on chromosome 7 and *Rd* (red pericarp) on chromosome 1 have been under-

stood, but their roles in regulating the flavonoids biosynthesis are unknown. The genes for dark purple pericarp formation, *Pb* and *Pp*, wait for finely mapping and functional characterization. In this field, MAS could be conducted to breed rice accumulating not only anthocyanins (a characteristic of black rice) and proanthocyanidin (a characteristic of red rice). Genetic transformation could be conducted to breed rice with accumulation of the anthocyanins or proanthocyanidin in the endosperm, since these phytochemicals accumulate only in the bran layer (Shao and Bao 2012).

5.3. Cooking and eating quality of brown rice

As concerns about nutritional quality rise, consumption of brown rice will become popular in the near future. Cooking and eating quality of brown rice will be another issue. The knowledge we have established for milled rice may not be applicable to the brown rice. Needless to say the genetic control of the cooking and eating quality of brown rice, what parameters to assess these qualities should be firstly considered. How to make brown rice appeal to consumers through suitable cooking methods should also be considered as well. At last, the question is how to improve the cooking and eating quality of brown rice.

5.4. MAS with more genes/QTLs together

Targeting more traits with more markers, such as *Wx*, *SSIIa*, and *fragrance* (Jin et al. 2010), is increasingly needed in the breeding programs. MAS for quality and yield and resistance traits should be considered together in the future. Strategies for more effective selection should be developed when many markers are used at the same time. *In silico* molecular breeding is coming into the era, with which alleles of different markers are designed in the computer; the phenotypes of new rice could also be designed and displayed in the computer.

6. Conclusion

Grain quality of rice as a whole is a complex trait that is comprised of appearance quality, milling quality, eating and cooking quality, and nutritional quality etc. Researches on the genetic control of the quality traits have made a great progress, especially for the appearance quality, cooking and eating quality. More genetic studies are needed for milling quality and nutritional quality.

The progress on the molecular genetics on grain quality has allowed MAS to be conducted more efficiently. However, only MAS for cooking and eating quality and genetic engineering for nutritional quality have made some achievements. More molecular breeding practices are needed for improvement of grain quality.

With social development and improvement of living standards, cooking and eating quality of brown rice will be a new theme that deserves greater attention from researches. Studies including cooking methods, parameters for cooking and eating, genetics, and molecular breeding are among the top priorities.

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